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Microbial Fuel Cells for Energy Production and Wastewater Treatment

Experimental studies

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Abstract

The exploration and creation of other ways to produce energy are becoming each day more important, since the use of fossil fuels have higher influence in the environmental degradation and they are not a renewable power source. Fuel Cells are a promising technology to produce electricity but further developments on this technology are needed before fuel cells can be introduced in the market. The Microbial Fuel Cells have a principle of operation similar to the other FC. The major difference is that Microbial Fuel Cells (MFC) use microorganisms at the anode side to convert chemical energy into electricity. The bacteria oxidize the organic matter and produce electrons. These electrons pass through an external circuit from the anode to the cathode and this flow originates electricity.

The combination of wastewater treatment with the production of electricity is the best advantage and the most interesting characteristic for the development of this technology. The wastewaters contain a lot of energy in the form of organic compounds and it could be used to create a sustainable cycle. Actually the MFC do not have the same grade of maturity and development of other technologies, which limits their scale up and represent one of the main disadvantages.

The main objective of this work is to study the effect of configuration parameters (membrane and electrode area and cell design) on the microbial fuel cell (MFC) performance. Were used a synthetic residual water simulating a effluent of the dairy industry and the bacteria used were *Lactobacillus pentosus*. Consequently, the performance of the Microbial Fuel Cell was evaluated based on the power density achieved, the efficiency in the chemical oxygen demand removal and the evaluation of the biofilm formed at the anode electrode.

The results presented in this work, show that operating a MFC in batch mode allowed to achieve better performances than in the continuous mode. Regarding the cell configuration, the use of a dual chamber MFC result in higher power outputs but the substrate degradation is higher with a single chamber MFC. The use of a lower membrane area allows a better performance but the wastewater treatment is more efficient with a higher area. Greater power densities were achieved by the utilization of a higher anode electrode but the COD (chemical oxygen demand) removal values achieved are lower than for the lower anode electrode size. For different amounts of yeast extract, the MFC reaches a better performance with the higher concentration, as well as, better substrate degradation.

KeyWords: Microbial Fuel Cells, Power output, Wastewater treatment, *Lactobacillus pentosus*, Biofilm.

Resumo

O desenvolvimento e criação de novas alternativas de produção energética tem-se tornado cada vez mais importante uma vez que o consumo de combustíveis fosseis tem uma elevada influencia na degradação ambiental e uma vez que estes não constituem uma fonte renovável de energia. As células de combustível representam uma tecnologia promissora para a produção de energia, no entanto, esta alternativa necessita de um maior desenvolvimento de modo a tornar-se uma opção real. As células de combustível microbianas (CCM) tem um principio de operação semelhante às outras células de combustível. No entanto, elas usammicroorganismos, no compartimento anódico, para converter a energia química em elétrica. As bactérias oxidam a matéria orgânica e, deste modo, são produzidos eletrões. Estes eletrões passam através de um circuito externo do ânodo para o cátodo e é este fluxo que é responsável pela produção de eletricidade.

A combinação do tratamento de águas com a produção de energia constitui a maior vantagem e a característica mais interessante para o desenvolvimento desta tecnologia. As águas residuais contem energia sob a forma de compostos orgânicos podendo, por isso, ser usadas para criar um ciclo sustentável. Atualmente, as Células de Combustível Microbianas não têm o grau de maturação e desenvolvimento de outras tecnologias, impedindo a sua aplicação à escala real, o que representa uma das suas maiores desvantagens.

Os objetivos principais deste trabalho são estudar a influência dos parâmetros configuracionais (área do elétrodo e membrana e configuração da célula) no desempenho das células de combustível microbianas, com vista à otimização das mesmas.Foi usada uma água residual sintética simulando um efluente proveniente da indústria do leite e foram usadas *Lactobacillus pentosus devido à falta de estudos com esta bactéria*. Consequentemente, o desempenho de uma Célula de Combustível Microbiana de foi avaliado com base na potência atingida, na eficiência de remoção da CQO (carência química de oxigénio) e avaliação do biofilme formado no ânodo.

Com a realização destes ensaios foi possível perceber que a operação da Célula de Combustível Microbiana em modo batch atinge um maior desemprenho do que operando em modo contínuo. Relativamente à configuração da célula o uso de uma câmara dupla resultou num pior desempenho, no entanto, a degradação de substrato foi superior aquando da utilização de uma única câmara. O uso da membrana com menor área permitiu a obtenção de um melhor desempenho, mas o tratamento de águas residuais foi mais eficiente para o ensaio realizado com a maior área. Maiores potências foram obtidas com a utilização do ânodo maior, mas as percentagens de remoção de CQO forem inferiores. Para diferentes quantidades de extrato de levedura, a Célula de Combustivel Microbiana atingiu o melhor desempenho para o ensaio realizado com dez vezes mais quantidade de extrato de levedura, tendo sido igualmente conseguida uma maior degradação do substrato.

Palavras-chave: Célula de Combustivel Microbiana, Potência, Tratamento de águas residuais, *Lactobacillus pentosus*, biofilme.

“Se é possível, é para se fazer...”

(Minae et Ambientalis Group)

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Contents

Nomenclature and Glossary

$(C_6H_{10}O_5)_n$- Amido

A - Membrane area (m^2)

BP-Brush

$C_6H_{12}O_6$- Glucose

$CaCO_3$- Calcium carbonate

C_e - Coulomb efficiency (%)

CO – Carbon Monoxide

CO_2- Carbon Dioxide

e^--Electron

Fe(III)EDTA - Ethylenediaminetetraacetic acid iron(III) sodium salt

H^+- Positive ion of hydrogen (proton)

H_2- Hydrogen

H_2O- Water

I – Current density (mA/m^2)

K_2HPO_4- Dipotassium hydrogen phosphate

KH_2PO_4- Potassium dihydrogen phosphate

$LiHO_2$- Lithium hydrodioxyde

$MgSO_4.7H_2O$- Magnesium Sulfate Heptahydrate

MRS – Bacteria Medium(based on the formulations of Man, Rogosa and Sharpe)

NH_4Cl- Ammonium Chloride

NO_x- Nitrogen oxides

P- Power density (mW/m^2)

R – Resistance (Ω)

Rpm-Rotations per minute

SO_x- Sulfur oxides

T - Temperature (ºC)

U (IV) – Uranium tetravelent

U (VI) - Uranium hexavalent

U- Voltage (V)

Acronymus

AFC- Alkaline Fuel Cells

ATP- Adenosine triphosphate

BMFC- Benthic Microbial Fuel Cell

CFU – Colony Forming Units

CHP- Combined Heat and Power

COD- Chemical Oxygen Demand

DMFC- Direct Methanol Fuel Cells

FC- Fuel Cell

HRT-Hydraulic Retention Time

HT- High Temperatures

LAB- Lactic Acid Bacteria

MCFC- Molten Carbonate Fuel Cells

MEC - Microbial Electrolysis Cell

MFC- Microbial Fuel Cells

NASA-National Aeronautics and Space Administration

OLR-Organic Loading Rate

PAFC- Phosphoric Acid Fuel Cells

PEM- Proton Exchange Membrane

PEMFC - Proton Exchange Membrane Fuel Cells

RFC- Regenerative Fuel Cells

SLR-Sludge Loading Rate

SOFC- Solid Oxide Fuel Cells

SRW- Synthetic Residual Water

USA - United States of America

VSS- Volatile Suspended Solids

WEC - World Energy Council

ZAFC- Zinc Air Fuel Cells

Greek Letters

α –constant (CFU/mg)

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1 Thesis Structure

This master thesis results from an experimental work developed with the aim of studying the performance of a Microbial Fuel Cell to produce electricity and simultaneously treat a wastewater.

This work is organized in six principal chapters. In this chapter the goal is to explain how this thesis is structured and which information contains each chapter.

The second chapter is subdivided in six sub-chapters. Initially a general introduction is presented regarding the main problems associated with energy and relating the importance of using alternative technologies to produce energy. The third sub-chapter includes the main operating principles of a generic Fuel Cell (FC) and presents a little description of the various types of FC and theiroperating principles. Like any other technology they have associated a set of advantages and disadvantages, these points and the main challenges to overcome are also described. After this generic part about the Fuel Cells, a description of Microbial Fuel Cells was addressed including their operation, materials, advantages and disadvantages, the applications and many other important subjects. In the final part of this chapter,some important issues about the dairy industry and about the microorganisms used in the experiments were also mentioned.

In Chapter 3, the parameters affecting the Microbial Fuel Cells performance are discussed in more detail and some of the studies carried by other authors in order to increase the knowledge about their operation are presented. The concerns on the scale-up of this technology are also described.

In the fourth chapter, a technical description of the experimental procedure performed during the experiments is provided as well as a description of the different materials and conditions carried during the tests.

Chapter 5 includes the experimental results obtained for the various configurations tested and a discussion about how this conditions affect the performance and the wastewater treatment of the Microbial Fuel Cell. Considering the tests performed in continuous mode, the effect of cell configuration, membrane area and electrode area on the MFC performance were studied. In the batch mode, studies regarding the effect of the yeast extract amount and the membrane thickness were carried out.

Finally in Chapter 6, the main conclusions of the present work are summarized and suggestions for future works are presented.

2 Introduction

The XVIII century was marked by big changes in industry and technology. The steam machines caused a new way of production with bigger revenues, less costs, faster, with a bigger amount of production and that caused an increase on the consume. All that contributed for the environmental pollution and other harmful consequences for the society. In a second stage of the revolution there was a bigger development on the electrical, chemical, metal and transport industry. The use of gas and oil was started as sources for energy generation, and the coal was being replaced (Infopédia, 2003-2014). The third phase started after the Second World War and remains until today. It is characterized by the use of a lot of energy sources, some older and other new, like oil, hydroelectric power, wind power, nuclear and many others combined with a bigger dependency of energy in every sectors. Differently of the Second Industrial Revolution, the Third Industrial Revolution, generates stationary power and creates fuel from renewable resources (Woodrow W. Clark, 2010).

The energy industry changed during the last two decades and the needs of energy grow faster than expected, and to this purpose other sources must be available.

As was said, during the last twenty years the world changed significantly towards a massive use of electronic and technologic devices contributing to a huge increase of electricity demand.

The United States of America (USA) are responsible for the consumption of one fourth of the total energy produced in the world and the Canada has the highest consume per capita. Countries like Brazil and China are increasing their consumption.

Nowadays, the energy demand is based on the exploration of fossil fuels as it is possible to verify in Figure 2.1. As the continuous power demand increases, the limitation of non-renewable resources and the rise of pollution is each day more important stimulating the use of new and renewable sources of power(Council, 2013).

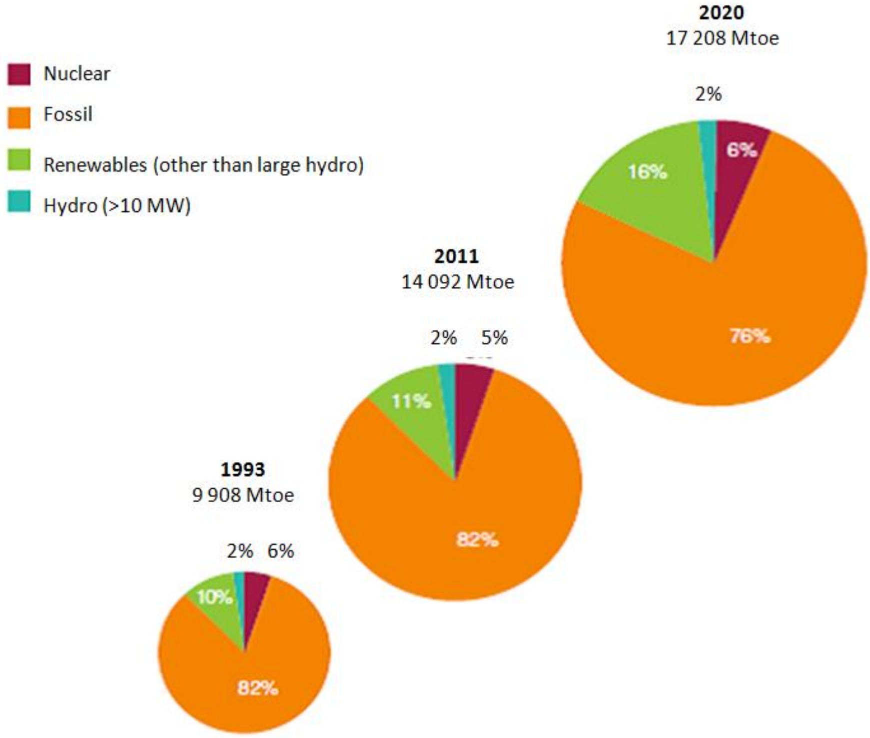


Figure 2.1- WEC Survey of energy resources in 1993 and 2011 and WEC World energy Scenarios for 2020(Council, 2013).

A lot of countries, like the USA and many others, must change and improve their fossil fuel generation and environmental degradation and advance to a politic of environmentally friendly renewable energy generation.

With the climate changes and the global warming interfering with our live every day,it is more important to include and link social and economic forces to create a sustainable future. In the last years, China is developing energy technologies and becoming leader on that. Almost all communities are capable to become energy independent and carbon neutral based on their renewable resources (Woodrow W. Clark, 2010).

Related with these issues, the technology and research have to be focussed on the development of alternative ways to produce energy. In the current world a lot of renewable resources are already working and giving a big contribution for the power production but there are, still, a lot to be improved. Local renewable power generation is required and distribution systems to avoid the losses and preferentially technologies with neutral emissions. The bet on renewable power sources has a big importance in the world nowadays and the development of other renewable technologies has been a concern. Fuel Cells are a promising way of electricity generation and a lot of studies have been made to turn this technology more efficient. However this alternative needs more investigation to become a real option.

This dissertation studies a specific type of Fuel Cell: the Microbial Fuel Cells.

2.1 Main goals

The main objective of this work was to study the influence of different configurations in the performance of a Microbial Fuel Cell and in the wastewater treatment. Different configurations were tested, starting by the effect of the operation mode, continuous and batch mode. The major part of the tests were performed in a single chamber MFC with the goal to optimize the operating conditions for this reactor, since the use of one chamber reactor presents some advantages, such as higher facility of operation and lower costs. Adding to that, a set of tests was performed with dual chamber configuration in order to compare the obtained results. The impact of the following aspects on cell performancewas studied: the cell configuration, membrane area and anode electrode area for MFC operating in continuous mode, and the yeast extract amount and membrane thickness for MFC operating in batch mode.

The performance of the Microbial Fuel Cell was evaluated based on the power density achieved, the efficiency in the chemical oxygen demand removal and the evaluation of the biofilm formed in the anode electrode, other parameters like pH, temperature and the number of colonies of *Lactobacillus pentosus* were measured in order to monitorise their variations.

2.2 Fuel Cells

Fuel Cells are a technology that generates electricity through electrochemical processes. This electricity is produced by the reaction between a fuel and an oxidant (usually oxygen from air). In this engine the fuel is not combusted which gives some particularly advantages, this allows to achieve a high efficient mainly if the heat produced by the reaction is used to heating. In theory, 83% of the energy of the fuel could be transformed in electrical power, a value much higher than most of the other technologies(Toyota, 2014).

According to their operation, it is possible to find many types of fuel cells. It depends on the temperature, the catalysts or the electrolytes. The procedure is similar to a battery because both generate electrical energy from chemical potential energy, and in both cases heat is produced.

The energy produced is a result of redox reactions (oxidation and reduction), in which oxygen is the oxidizing agent and the fuel is the reducing agent.

Each cell is composed by two electrodes, one positive and other negative, one electrolyte between both electrodes and a catalyst. On the electrodes occur the reactions responsible for the production of electricity, the electrolyte is responsible for the passage of ions from one electrode to another and it is selective, which means that only some ions are capable to pass through it. This is important because other substances could influence the chemical reaction and reduce the cell efficiency. The catalyst is used to accelerate the reaction (Russo, 2014; FuelCells2000, 2014).

The main constitution of a Fuel cell is represented in the Figure 2.2.

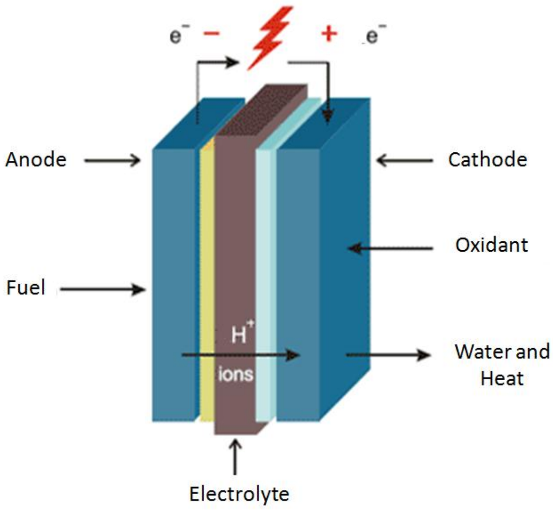


Figure 2.2-Schematic representation of a Fuel Cell, Adapted from:(Erik Kjeang, 2012).

In Fuel Cells, the fuel is provided to the negative electrode where the reaction occurs and the electrons and ions are release. These electrons go from the negative electrode to the positive electrode creating a flow and generating electricity. The ions pass through the membrane to reach the positive side, and join to the oxidant to form water(Toyota, 2014).

The process could be divided in three steps as described on the Figure 2.3:

1. Supply of fuel to the anode. Oxidation of the fuel and formation of protons (H^+). They will pass through the electrolyte and electrons that will go to the external circuit.

2 . Reduction of the oxidant on the cathode caused by the reception of electrons from the external circuit.

This charges flow will produce electricity.

3 . The water is formed by the link of positive ions and negative ions originated on the reactions that occur in the electrode interface.

This process happens in the electrolyte.

$$Fuel + Oxidant \rightarrow Water + Electrical\ power + Heat$$

Figure 2.3 - Electrochemical process of a Fuel Cell, Adapted from(Martins, 2003).

In some big companies this type of technologies are starting to be applied. Some are combining Fuel Cells with other technologies to support the power needs and reach their sustainability goals (Jennifer Gangi, 2012). Fuel Cells have a big advantage because they do not release pollutants, so the companies and industries can produce a bigger amount of electricity without the emission of thousands of metric tons per year of carbon dioxide. Fuel Cells can easily be sited in every place which allows a flexible and portable planning. They are long lasting and each cell operate according to their operational characteristics, providing advantages to particular applications and making this technology very versatile (Russo, 2014). As already mentioned, the way of operating is similar for every Fuel Cell, they could differ in the electrolyte material and in the range of temperatures and consequentlyon the cell performance. The different types of Fuel Cells (FCs) are: Proton Exchange Membrane Fuel Cells (PEMFC), Direct Methanol Fuel Cells (DMFC), Alkaline Fuel Cells (AFC), Phosphoric Acid Fuel Cells (PAFC), Molten Carbonate Fuel Cells (MCFC), Solid Oxide Fuel Cells (SOFC) and other like Regenerative Fuel Cells (RFC), Zinc Air Fuel Cells (ZAFC) and Microbial Fuel Cells (MFC).

2.2.1 Proton Exchange Membrane Fuel Cells (PEMFC)

This type of Fuel Cell has high power density and operates in a range of temperatures between 79ºC and 93ºC. The electrolyte is a solid polymer membrane, water-based, and they are used when a quick startup is required to power applications and could be adapted for the electrical output meet dynamic power demands.

A single PEMFC could reach several watts to several kilowatts. These type of cells are commonly used in prototypes of vehicles and their efficiency rounds the 40-60%. The fuel used could be hydrogen, methanol or other reformed fuels. The platinum is the better catalyst for fuel cells that operate at low temperatures.

This FC could also work at higher temperatures (HT PEMFC), starting in 121ºC until 199ºC. The electrolyte is changed to a mineral acid-based system. They are also capable to process fuel containing small quantities of carbon monoxide (CO). Applying fuel reformers it is possible to usea wider amount of input fuels (FuelCells2000, 2014; Russo, 2014).

2.2.2 Direct Methanol Fuel Cells (DMFC)

The electrolyte used is the same used PEMFC and the catalyst used is platinum joined with ruthenium in the anode side. They operate at relatively low temperatures, between 51ºC and 121ºC. The characteristics are similar to the first type of Fuel Cell described but in DMFC it is not necessary a fuel reformer because the anode is capable to take hydrogen from liquid methanol.

The range of temperatures and efficiency higher than 40%, make these FC interesting to be used in portable applications like cell phones, battery chargers and others small gadgets. The methanol has high energy density and could be easily transported and stored. On a fuel cell unit the alcohol could be supplied by a liquid reservoir that could be quickly switch (FuelCells2000, 2014; Russo, 2014).

2.2.3 Alkaline Fuel Cells (AFC)

The range of temperatures is higher, operating around the 107ºC and 246ºC and is commonly fuelled with hydrogen. At the same time the electrical efficiency is considerable high, reaching the 60-70%. They could operate with small quantities of CO_2 and due to that this type of FC is attractive to aerospace and underwater environment. They were used to produce electricity and water in missions on the space developed by NASA.

The electrolyte used is a potassium hydroxide solution in water and different non precious metals can be used as catalysts, for example nickel that is already used (FuelCells2000, 2014; Russo, 2014).

2.2.4 Phosphoric Acid Fuel Cells (PAFC)

The electrical efficiency of this FC is not very high reaching values only around 40%. The electrolyte is composed by a liquid phosphoric acid ceramic in a lithium aluminum oxide matrix and the catalyst is a carbon supported platinum. The reactions between electrodes are similar to the reactions in PEMFC but the range of temperatures is 177-204ºC. One vantage is that they are more tolerant to the impurities in fuel. PAFC are already sold for systems that have a high energy demand like wastewater treatment plants, hospitals, buildings and industries. Before 2001 the most fuel cells sold were PAFC (FuelCells2000, 2014; Russo, 2014).

2.2.5 Molten Carbonate Fuel Cells (MCFC)

The electrolyte is a ceramic matrix of $LiHO_2$ with alkali carbonates and the catalyst used is normally non platinum. These Cells operate at very high temperatures, 649ºC and because of that the hydrocarbon fuel could be converted to H_2. They are tolerable to carbon oxides so they can operate with a large type of fuels like methane, natural gas and some coal derived fuel gas, without using external reformers.

These Fuel Cells are appropriate to large stationary power and CHP applications, they can also be used in systems with an energy demand of megawatt capacity. The electrical efficiency rounds the values of 50% to 60% and when the heat is recovered the efficiency rounds 80%.

One disadvantage of these cells is the liquid electrolyte and the necessity to inject carbon dioxide at the cathode (FuelCells2000, 2014; Russo, 2014).

2.2.6 Solid Oxide Fuel Cells (SOFC)

The efficiency of these cells is about the same of the MCFC. They use a solid ceramic as electrolyte and the catalyst is non platinum also. The operating temperature is around 900ºC which allows internal reforming of light hydrocarbons without an external reformer and with the possibility of using different fuels.

They have two different configurations: one uses compressed discs and other an array of meter long tubes. Some can be used for big stationary applications and other at vehicle auxiliary power units or smaller applications such as houses.

The main problem of SOFC is the time needed to start up and to reach the operating temperature (FuelCells2000, 2014; Russo, 2014).

2.2.7 Regenerative Fuel Cells (RFC)

These Cells use an electrolyte powered by solar energy to separate the water in hydrogen and oxygen. These elements are supplied to the FC and then all the process occurs like the basic FC. The water produced is re-circulated to the first electrolysis stage (FuelCells2000, 2014).

2.2.8 Zinc Air Fuel Cells (ZAFC)

In this FC the electrolyte is combined with zinc pallets and air. The process generates electricity and it is possible to get more power than in the lead-acid batteries (FuelCells2000, 2014).

2.3 Advantages and Disadvantages of Fuel Cells

The development of FCs has been slow mainly due to economic reasons and to the difficulty in understanding the complexity of the kinetics occurring on the electrodes.

One disadvantage of FCs that works with hydrogen is the fact that this element is not in the pure state on the nature, so it is necessary to extract it from other fuels rich in this compound. As mentioned before, in some types of Fuel Cells a previous treatment of the fuels before use is necessary . This first step releases carbon monoxide and carbon dioxide and for this process high temperatures are required (Martins, 2003).

The continuous use of Fuel Cells can decrease their performance due to the degradation of some properties. The corrosion of the carbon on the electrode, depending on the type of carbon used and the water steam pressure contribute to a decrease in the cell activity.

At the same time, the continuous use of the FC is an advantage because it works without interruption much more time than the vast majority of the technologies actually used (Santos, 2004).

The cathode can be coated with some elements or substances in order to increase the cell performance such as phosphoric acid (in the case of using platinum). The adsorption of impurities can negatively influence the activity of the FC (Martins, 2003).

This technology is very efficient and can produce two times more energy than a conventional machine transforming calorific energy in mechanical energy. Adjusting the number of FCs is possible to achieve the desired power generation. These characteristics are very useful in equipment sensible to variations in voltage and current.

As was mentioned, a big advantage of the FCs and maybe the main motivation to continue the development of this technology is their near zero emissions, since they do not release pollutants like NO_x, SO_x, CO, and hydrocarbons to the atmosphere. The use of these cells eliminates or reduce the consumption of fossil fuels and consequently the emission of greenhouse gases. When hydrogen is used, the reaction products are, only heat and water (NationalFuelCellResearchCenter, 2013).

Another point is the fact that Fuel Cells are very quiet and this stimulates consumers to have electricity generation near home without problems. The use of this technology increases the reliability, increases the efficiency by the reduction of the distance source-consumer and reduce the time to answer costumers needs.

The efficiency, depending on the Fuel Cell type and design, is between 30% and 60%. Using this system in process of co-generation it is possible to achieve efficiencies near the 70% and when the heat produced is used it is possible to achieve an efficiency of 85%. Compared with traditional technologies the value of efficiency is much higher (NationalFuelCellResearchCenter, 2013; Santos, 2004).

FC stacks can be build-up with FCs units and in this way they can be easily moved or transported in a short time. Even if they are not need any more in a place they can be moved to another.

The price of this technology, as well as, the research costs, the production processes and the materials used are actually the main obstacle to their implementation (Santos, 2004).

2.4 Microbial Fuel Cells

The principle of Microbial Fuel Cells is quite similar to the other Fuel Cells but with some specific particularities. Microbial Fuel Cells use some microorganisms to convert chemical energy, available in the organic matter, into electricity (Liew et al., 2014; Singh Mathuriya, 2013). Through redox reactions the bacteria will degrade (oxidize) the organic matter and produce electrons. They pass through a set of respiratory enzymes and produce ATP which is the cell energy form. Then the electrons pass to the terminal electron acceptor where the electrons are reduced. This flow of electrons to final acceptor, will originate electricity (Logan, 2008). Basically they produced electric energy athwart the bacteria metabolism. The main goal and at the same time the main advantage is the organic matter degradation combined with the electricity production. The configuration of a MFC is represented in the Figure 2.4:

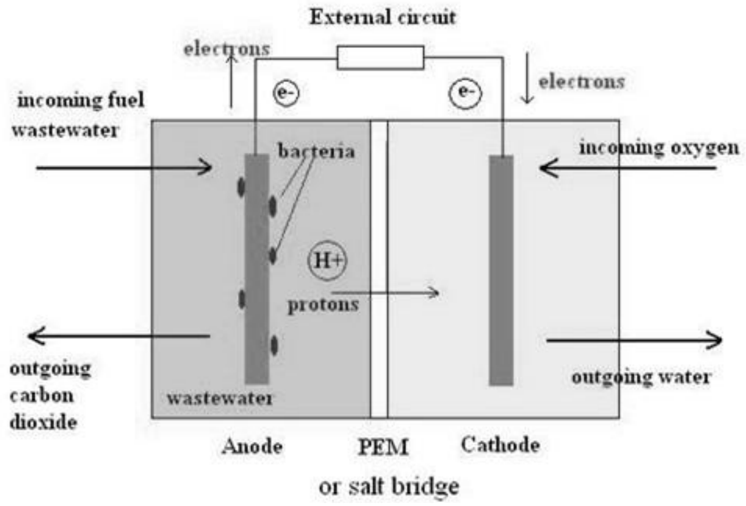


Figure 2.4- Configuration of a two chamber Microbial Fuel Cell, Adapted from (Maranowski, 2013).

Every MFC is constituted by an anode and a cathode separated by a proton specific membrane, as the generic Fuel Cells. These Cells could be composed by two chambers (one for the anode and another for the cathode) or by a single chamber where the cathode is side by side with the membrane as represented in the Figure 2.5:

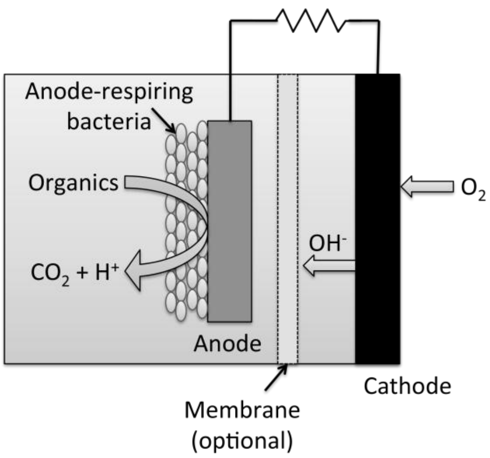


Figure 2.5- Configuration of a single chamber Microbial Fuel Cell, Adapted from (Kenntron, 2013).

The electrons and protons are originated by the oxidation of the substrate - our fuel - through the bacteria in anode chamber.

It ispossible to operate a MFC with different types of substrates, from simple organic effluent to complex wastewater (Singh Mathuriya, 2013).

Once the oxygen is the terminal electron acceptor is not good if it is available in the anode chamber because will avoid the passage of electrons to the cathode. Because of this it is necessary to separate the anode (bacteria and substrate) to the cathode (catholyte and oxygen) and this is possible with the use of a membrane. This membrane is load permeable so the protons could pass to the cathode and form water by the combination with oxygen. This reaction could be demonstrated by the following equation (Singh Mathuriya, 2013):

$$4H^{+} + 4e^{-} + O_2 \rightarrow 2H_2O \tag{1}$$

In the anode, once that oxygen is not available the electrode work as the acceptor of electrons and after that they pass through the circuit to reach the cathode. In this compartment of the cell the microorganisms and the organic matter that will be degraded are presented. These characteristics will be discussed in the following sub-chapters.

2.4.1 Voltage and Power generation

The voltage values obtained with MFC are not very high and the highest voltage produced by the MFC can be measured when the circuit is opened (not connected) and therefore the current is null and the resistance is maxim. The voltage depends on the current and the external resistance (R) (circuit load) as follows (Logan, 2008).

$$U = I \times R \tag{2}$$

The power of a Microbial Fuel Cell is characterized as the product of the current with the voltage.

$$P = I \times U \tag{3}$$

Normally the power is normalized by the surface area of the anode to express better the efficiency of the system according with the design of the MFC, in some cases this normalization is based on the surface area of the membrane (Logan, 2008).

In order to improve the power generation it is necessary to achieve the best values of the two parameters. For reach that goal is also necessary to extract from the biomass most of the electrons as current. The fraction between the electrons available on the initial organic matter by the electrons that were recovered to produce current is defined as Coulombic efficiency.

$$C_e = \frac{Coulombs\ recovered}{Total\ coulombs\ in\ the\ susbrate}$$

Basically the energy efficiency is the energy that was recovered by all the energy available initially in the system.

To maximize the power production it is needed the smallest difference in voltage as the increase of the current.

The voltage as a function of current density could be represented by a polarization curve, as represented in the Figure 2.6.

The electrochemical reactions between the organic matter and the final acceptor influence the MFC performance. The top of this curve give us the major power reached by the cell. Anyway, as can be seen, the ideal performance is smaller than the theoretical value calculated for the cell (Logan, 2008). It is due to three basic types of irreversible losses: activation, ohmic resistances and by mass transport (or concentration).

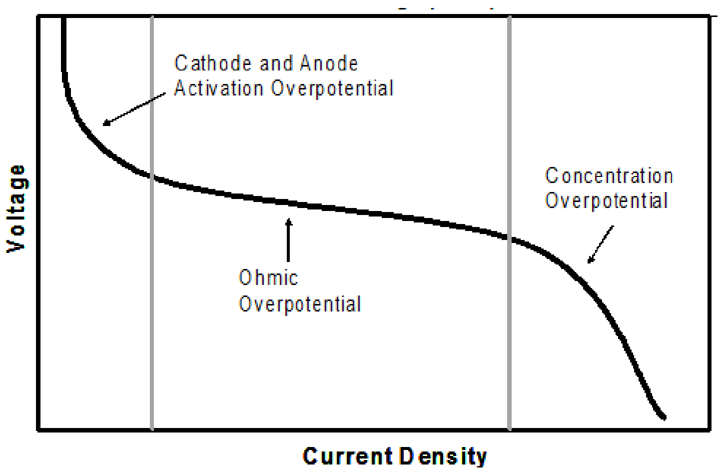


Figure 2.6 - Polarization Curve(Yeetsorn, Fowler, & Tzoganakis, 2011).

In the polarization curve it is possible to analyze three regions/behaviors of the voltage. In a first stage occur a quickly fall of the voltage while the current pass through the circuit. In a second stage occur a linear decrease of the voltage and in a third stage occur a rapid decrease at high values of current density.

Activation losses are caused by the loss of energy provoked by the initiation of the reactions of oxidation and reduction and by the passage of electrons from the cell to the anode surface. Also the nature of the electrode surface account to this loss. In other words, the reacting species must overcome the activation energy and this provokes a voltage decrease.

The use of cathode catalysts or increasing the electrodes area could decrease these losses. With the addition of mediators, the energy required for the release of electrons by the microorganisms to the anode is minimized (Oliveira at al., 2013). At low current densities the activation losses are dominant.

Bulk phase could cause some mass transfer resistances but it is called as ohmic resistances. These **ohmic resistances** represent a very important parameter to be overcome. It corresponds to the resistance to the passage of electrons and protons in the various compounds of the cell. For the electrons the passage through the electrode, for protons the resistance is due to the selective membrane and in general some internal connections. Basically, is due to ionic and electronic conduction (Logan, 2008; Pinto, 2014; Oliveira et al., 2013).

These types of losses can be reduced with the rearrangement of some components of the cell such as decreasing the distance between the electrodes and increasing the ionic conductivity of the electrolytes (Logan, 2008; Pinto, 2014; Oliveira et al., 2013).

The **mass transfer losses** or **concentration losses** correspond at phase when the products or the reactants are not enough and limit the reaction. This means that exist incapacity to maintain the substrate concentration.

In some cases the substrate flow is a problem to MFC operation, the proton accumulation could cause a decrease in pH affecting the bacteria. Proton transfer to the cathode can be limited by mass transfer and could also limit the production of electricity (Logan, 2008).

Once the polarization curves describe the losses behavior, the analysis of each part could help to minimize them and improve the MFC performance. This could be avoided using mediators and doing modifications in the MFC configuration, as the space between electrodes, the electrode material, the catalysts, membrane area and changes in other components.

2.4.2 Advantages and disadvantages

This technology offers a lot of advantages when compared to many others technologies. The combination of wastewater treatment with the production of electricity is for shore the best advantage and the most interesting characteristic for the development of this technology. This combination is capable of decreasing the final cost of the process (Singh Mathuriya, 2013).

The MFCs can act as anaerobic reactor. When compared with others processes of wastewater treatmentthis does not use much energy. By the contrary,it can provide energy to the system. Or if the energy amount produced is sufficiently high the process of operation could be energetically sustainable (Rahimnejad et al, 2011).

The wastewaters contain a lot of energy in the form of organic compounds and it could be used to create a sustainable cycle, doing the wastewater treatment and thereby produce enough energy to cover the expenses.

They have also an advantage about thermal machines because the electrochemical process does not have the same limitation that processes based on the Carnot Cycle (Gomes, 2001).

The gas mainly produced is carbon dioxide (CO_2) but in this process will not be produced more than in other kind of treatment processes. At the same time, avoiding the use of fossil fuelswill translate a reduction of carbon dioxide emissions. Once the production of gases is not significant it is not needed gas treatment (Singh Mathuriya, 2013)

When compared with another type of Fuel Cells the fact of be fuelled by organic matter is a good advantage because it is not toxic as methanol or explosive as hydrogen(Oliveira et al., 2013).

Actually the MFCs don't have the grade of maturity and the development of others technologies and this is one of the main disadvantages of this technology (Logan, 2008).

The cost associated at the fabrication and the materials of this technology has to be more competitive. Actually, the most common catalyst used on the cathode is platinum which constitutes a big part of the capital cost of the MFC (almost 50%) (Liew et al., 2014).

Despite all the advantages, the power densities achieved with this technology are lower when compared with other technologies so it is difficult to make it more competitive. However, much research need to be been done in order to improve and make this technology more attractive.

2.4.3 Applications of Microbial Fuel Cells

As mentioned, the application most spoken for this technology is the treatment of wastewater rich in organic matter and consequently an alternative technology to energy production. This application could be reflected in a lot of applications in many industries or wastewaters plants. As Logan said "While the energy that could be capture from wastewater is not enough to power a city, it is large enough to run a treatment plant." (Logan, 2008). The use of the MFC for wastewater treatment instead of another treatments reduce the production of solids, reducing also the operation costs for their handling and eliminate the costs of aeration once the degradation in the MFC occurs under anaerobic conditions (Logan, 2008).

The Microbial Fuel Cells can be used for other purposes. In some cases the electricity produced is not enough to become economic feasibly but it could be use to powering some devices (Kenntron, 2013). Normally the monitoring parameters are transmitted to remote receivers that are commonly powered by batteries. The microbial Fuel Cells could be used to provide power to these sensors and to remote receivers. This application avoids problems of limited lifetime and replacement. To have the necessary power to supply these devices, the energy produced by the MFC was stored in a capacitor (Shantaram et al., 2005).

The MFC could be designed for take the potential generated by the bacteria from the oxidation of organic matter to produce hydrogen gas instead of electricity. This could occur by the addition of a bigger amount of voltage, 0.23 V, to the power density produced in the anode and excluding the oxygen in the cathode. Normally the name given to this technology is MEC. Once this is a relatively new technology the investigation and developments is not much detailed. As Logan reported were used acetate as a substrate and a domestic wastewater (Logan, 2008).

The MFC could be also used as remote source of power for devices in the sea surface or for devices operating in underwater environment. This technology implies the use of the organic matter and the anaerobic conditions in the floor sediments of the sea to operate the anode and place the cathode in the overlying water in order to be in contact with the dissolved oxygen. The first tests were carried out by Reimers et al. in 2001, reaching 15 mW/m^2 using platinum anodes, followed by a set of new tests with another materials. The main problems of this application are the limitation provoked by the organic matter concentration in the sediments and the rate of degradation support by the bacteria.

The MFC could, also, be used for bioremediation. This method consist in add a long-lasting substrate to the anode, like chitin, as an example, to produce power and then the bacteria use the electrons in the cathode to reduce some compounds as nitrate or U (VI). Gregory at al. in 2004 was the first to explore the utilization of biocathodes for the bioremediation of nitrate. The nitrate could be converted to nitrite by applying an extra potential to pure cultures of *Geobacter* species that use the electrons from the cathode to do it. The same method, using a cathode was used by Gregory and Lovley in 2005 to do the reduction of U (VI) to insoluble U(IV). A *Geobacter* specie was add to the cathode and U (IV) was deleted from the solution since the system remains in anoxic conditions. This method could be used to remove uranium from groundwater and accumulate it in the electrode. Is also possible to occur bioremediation in the anode when the concentration of biodegradable organics is high and exist a big amount of electron acceptors presents in the cathode. In 2007 was made a study with a petroleum-contaminated groundwater and was achieved a high power density by the biodegradation of petroleum compounds.

Another application of this technology is their use for Chemical Oxygen demand monitoring for in situ pollutants(Pinto, 2014).

Lately, some people are using these cells to study exoelectrogenic microorganisms and the exoelectrogenic biofilm formed in the anode surface(Logan, 2008).

2.5 Dairy Industry

In the case of this study it was used a synthetic effluent of a dairy industry once that is a very expressive type of industry in Portugal. Another point that contributes to that was the fact that was possible to use *Lactobacilus Pentosus*, a type of bacteria used in these industry and which shows a good capacity to degrade the organic matter.

Commonly a high amount of waste and food waste originated in industrial activities is rejected to the sewage drains. The dairy industry is one of the leader industries of food-based of the world and they produce a significant amount of wastewater (Singh Mathuriya, 2013).

In these industries the milk could be processed for two main reasons: production of consumer milk or derived products like yogurts, butter, cheese, butterfat, milk powder, condensed milk and others.

The water consumption and the production of wastewater have a big expression in this kind of industries in many operations as heating, cooling, equipment and installation washing. The process is composed by a set of main operations like clarification operations, separation, pasteurization, coagulation and incubation. According to the size and possibility of the company, some industries apply a pre-treatment or wastewater treatment, before discharge such as filtering with activated carbon or sand filters and disinfection using chlorine or ultraviolet radiation (Secretaria Regional do Ambiente e do Mar, 2011).

During the industrial process the water washing of equipment and installation drag the wastewater have a big amount of organic compounds (Singh Mathuriya, 2013). This fact is one of the main environmental concerns of the process. The operations that contribute more for the production of wastewaters are: liquid that results of the cheese production, the losses during discharge operations and filling, wrong utilization of the equipment or lack of maintenance that result in leakages, discharges during the starts and stops of the equipment and the change of the products, the tanks washing at the receiving site and the waste product that remains in the machines components (Secretaria Regional do Ambiente e do Mar, 2011).

2.6 About the use of *Lactobacillus*

In these type of industries it is commonly used *Lactobacillus*. These microorganisms are added to milky products because they produce lactic acid and antimicrobial substances. This help to control the quality of the product and avoid the development of some microorganisms that could degrade the lactic product. The Lactic acid bacteria (LAB) like *Lactobacillus* have a very significant paper on fermented foods production because they contribute to the acidification especially in the manufacture of cheeses, at the same time, LAB could increase the quality of the final product (Bautista-Gallego, et al., 2014).

These microorganisms use the fermentative process to oxidize the organic molecule but not all the energetic potential is extracted. The product of this process depends on the initial substrate used and could be organic acids, alcohols, acetones and gases.

As mentioned the big concentration of organic compounds in the dairy wastewater is a problem and need some treatment to be possible reject it in environment. The problem could be solved or attenuated with the use of some microorganisms by a process that could be close to bioremediation (Nunes, 2007).

In our case we used bacteria in pure cultures constituted only by *Lactobacillus Pentosus* and these bacteria provoked an alteration in the oxidation state of the “pollutant”. The effluent was used as a source of energy.

The use of this bacteria is very good because they can replicate by themselves, once they have organic matter, so the process could occur almost in a self-sustainable form.

According with the type of microorganism it is possible that the reactions take place between a large range of temperatures (Nunes, 2007; Logan, 2008).

3 State of Art

The Microbial Fuel Cell study and development was mainly explored in the recent years. However in many places as United States of America, Japan and in Europe it was already explored.

In 1911, Michael Cresse Potter did some studies about the oxidation of glucose in the anode of a Fuel Cell using the bacteria *Escherichia Coli an Saccharomyces* using electrodes of platinum(Gomes, 2001; Pinto, 2014).

Luigi Galvani was the first to study the metabolic process in live organisms and electricity when observed the electricity production in the legs of the frog (Remya at al.).

Since that data the technology was not much discussed, returning to be explored and becoming popular in the 60s. In that time the National Aeronautics and Space Administration (NASA) began to study the transformation of organic matter into electrical energyto take advantage of organic waste produced in space travel(Gomes, 2001).

Thereafter started to be developed a wide set of tests principally from the 70s. In this decade was tested a MFC using *Clostridium butyricum* to produce hydrogen by the glucose fermentation.

Kim in the 90s used a Fuel Cell as a method of determining the concentration of lactate in water by the using a bacteria (Logan, 2008). In 1991 the MFCs were used for the first time to treat domestic waste from that, this has been the most explored purpose of Microbial Fuel Cells. In 1999 was discovered that mediators did not need to be added (Logan, 2008).

In 2004 was demonstrated that domestic wastewater could be treated and at the same time it is capable to generate electricity (26 mW/m^2).

Rabaey et al. demonstrated that was possible to reach power densities much higher (two orders of magnitude more) using glucose as substrate in a MFC without the use of mediators (Logan, 2008).

The evolution of this technology has been described in a large number of articles and the results are very variable. During the description of the several compounds and conditions of our experimental work it will be appointed the various variables used by us and studies performed by others authors and in some cases compared with our results.

A lot of factors affect electrons transfer to the electrodes and the fuel oxidation, influencing the performance of the Microbial Fuel Cell. Also, the wastewater treatment, evaluated by the COD removal, is influenced by a set of parameters. Among these parameters are included the substrate concentration, the microorganisms used, the hydraulic retention time, the coloumbic efficiency, the oxygen consumption rate, the pH, the temperature, the circuit resistance, the proton transport to the cathode through the PEM, the biofilm, the cell configuration and operation mode (Chen et al., 2014; Rahimnejad et al., 2011). In recent years the work in MFC has increased, either in microbiologic issues as configuration and operation problems in order to increase the power densities. The study of the effect of the various parameters appointed above is the way to improve the MFC performance and know what and how is possible to do it.

Relatively to the electrodes used on the anode side it is necessary to test or develop new surface materials in order to enhance the biofilm adhesion and study surface properties with greater potential as electron acceptor. Develop cathodes with higher electrochemical potentials and increase the velocity of electron transfer between both electrodes is also needed. Lana et al.(2014), studied the influence of the anode brush diameter, the number of anodes and the space between electrodes in the MFC performance. They found that larger brushes achieved a better performance than a higher number of smaller anodes. Also, shortening the distance between the smaller electrodes, even only a few millimetres, results in a higher power production, the same does not occur with the higher brushes (Lanas et al., 2014). Huang et al. (2008) tested another type of materials to the anode electrode. They performed tests with graphite-fiber brush anodes in order to study its performance since it present low resistance and the organization of the filaments provide a higher brush porosity. The space between the electrodes is also a very important parameter, Liu et al. (2008) found the spacing between the anode and the cathode electrodes were more important than the reactor size (Huang et al., 2008).

Related with the semi-permeable membranes or other proton exchange media is required to explain how is possible to increase the proton transport and at the same time reduce the possibility of transferring electron acceptor from the cathode to the anode.

The questions and problems about the microbiology part of the MFC are very important and at the same time complicated because the behaviour of it in many cases are not much predicted and depends of the microorganisms and a lot of others parameters. Some of the main questions are if is possible to isolate bacteria that grow on non expensive substrates, if they had the capacity to produce more electrons and do a faster transference to the anode and if it is possible to form biofilms with higher capacity to promote bigger potentials with some cultures.

About the wastewater treatment the main question is if the MFC are capable to present treatment results that compensate their low electricity production and if this technology is capable of being used in some kind of treatment plants.

Concerning the biofilm it is important to know more about the variables that affect its structure and composition with the purpose of increase the electron transfer.

All the operation conditions, such as temperature, pH, feed rate or the aeration rate, between others, require a deeper knowledge in order to know better how they affect the cell performance

3.1 Mediators

In the beginning the power output achieved with MFC was very low. Only when was discovered that this values could be increased by the addition of electron mediators it became more interesting.

Since the studies made by Potter in 1911 with *Escherichia coli* and the yeast *Saccharomyces cerevisae* resulting in voltage production a lot of studies and experiments have been done to improve the electrons transfer between the electrodes. Some of these mediators are the potassium ferricyanide, methyl viologen, thionin, neutral red, methylene blue, Fe(III)EDTA.

Sometime after, Rabaey in 2005 show that mediators do not have to be add to the culture once some bacteria like *Pseudomonas aeruginosa* were capable to self produce a chemical mediator and in this way transfer the electrons from the anode to the cathode and produce electricity (Logan, 2008). Also this showed some problems in the accumulation of amounts required to maintain or achieve high concentrations. Only some types of bacteria are capable to produce electricity without the addition of exogenous mediators like *Geobacter* and *Rhodoferax spp.*, they are called electricigens because they are capable of transferelectrons directly to the electrode and increase the Coulombic efficiency (Oliveira et al., 2013). More studies were made with *Shewanella species*, *Aeromonas* and *Geobacteraceae* and showed power production, even if small. In any case the addition of mediators caused an increase of the power output at least of 30% (Logan, 2008).

The exogenous mediators must be capable to easily pass the cell membrane, have a high electrode reaction rate, be non-toxic to the bacteria, be non-biodegradable and do not have a high cost. Normally their cost, toxicity, instability and not higher efficiency limit their use.

3.2 Effect of pH

The pH is an important parameter because it can influence the activity of the microorganisms in the substrate and affect the production of electricity. A neutral pH is the ideal condition for an optimal bacterial growth. The pH alterations induce changes in the ions concentration, membrane potential, proton-motive force and in the formation of the biofilm. Also the reactions that occur in the MFC cause an alteration in the pH. If a slow or incomplete proton diffusion exists, an accumulation of protons and consequently a migration through the membrane occurs inducing a decrease in the pH of the anode. This variation will cause a decrease on the activity of the microorganisms and consequently will affect the performance of the cell. Also the continuous combination of protons with oxygen at the cathode contributes to an increase of the pH at the cathode side. According with Nernst equation, this results in a potential decrease since the reduction of oxygen leads to a pH increase. To avoid this, it was studied how buffers can maintain the values of pH at the anode and the cathode. Because of their chemical composition and their interference with bacteria, electrodes and membrane, the buffers are capable of reduce the changes in the effluent pH and in the biofilm. Phosphate, bicarbonate, zwitterionic and borax are being used as buffers but in some cases their use have big disadvantages since it is necessary to remove them from the effluent before proceed to the discharge, increasing the costs of this technology (Oliveira et al., 2013).

Some studies were performed in order to test the addition of buffers in the cathode side without affect so much the ohmic resistances and the power outputs. The use of saline solutions was tested and presented good results for control the pH, but it can affects the bacteria in the anode compartment (Oliveira et al., 2013).

3.3 Effect of temperature

High temperatures can increase the MFC performance since it benefits the electrochemical kinetics occurring in the electrodes, increase the open circuit voltage and reduce the activation overvoltage. It also increases the conductivity of the solution with a consequent reduction of the ohmic resistance. However, the effects are not only positive, once it can influence the stability of the membrane and cause a decrease of the partial pressure of the oxygen.This parameter has a particular importance in the MFC because of the microorganisms and could affect its growth rate. According with the bacteria used the optimal temperatures are different and it affect the intracellular biochemical process, the extracellular chemical process and the bacteria metabolism rate (Oliveira et al., 2013).

Some kinds of microorganisms are more sensitive to temperature variations than others. Studies have been performed in order to understand the effect of the temperature on MFC and until now it was discovered that the MFC presents better performances when the operating temperature is between 30ºC and 40ºC. Operating at higher temperatures cause an increase in the start up time when compared with the operation at lower temperatures. It can also cause a decline in cell function provoked by damages in important elements of the cell, such as nucleic acids and proteins. Instead of increasing the anode temperature, an alternative could be increasing the cathode temperature to avoid the problems related with the temperature effect in the bacteria growth and metabolism (Oliveira et al., 2013).

3.4 Organic load

Since the performance of the Microbial Fuel Cell depends on the production of electrons by the microorganisms and the oxidation of organic compounds release protons and electrons, the organic load is a very important parameter to have into consideration.

The studies carried out take into account two parameters in order to know the effect of the organic load in the MFC performance. These parameters are the organic loading rate (OLR), related with the conversion of organic compounds per reactor volume, and the sludge loading rate (SLR), related with the capacity to convert organic substrates per mass of bacteria. Both have an important influence in the potential achieved, the chemical oxygen demand removal and the coloumbic efficiency (Oliveira et al., 2013).

As already mentioned, if more organic matter is available, higher will be the microorganisms activity and consequently greater will be the power density achieved. So, an increase in the OLR will increase the cell performance and was also discovered that with this increment the internal resistances decreases (Logan, 2008; Oliveira et al., 2013).

However, if the organic loading rate is excessively high it will cause an excessive nutrient supply that will not be used to electricity generation provoking a decrease on power generation. Higher OLR values lead to a competition between bacteria that will lead to a higher organic matter removal that cannot be related to current generation. So it is possible to occur an increase in the substrate degradation combined with a coloumbic efficiency decrease (Oliveira et al., 2013). It is necessary to find an optimal value to the OLR that increases the power generation and at the same time increases the coloumbic efficiency.

3.5 Feed rate and shear stress

The biofilm formed on the anode electrode has a high importance in the electrochemical activity and in the capacity of the MFC to treat a wastewater. The hydraulic conditions affect the formation and the adhesion of bacteria to the electrode, avoiding or helping the creation and development of the biolfilm. Pham et al. (2008) realized that the potential could increase two or three times with an increase in shear stress. In these conditions the biofilm formed presents the double of the thickness and the density of the biomass increased five times. This happen since the biomass is subject to greater detachment forces and in answer to that the biofilm creates higher cohesion between particles resulting in a higher electron concentration and consequently in a higher potential. Nonetheless, if the shear stress is very high the microorganisms attached to the anode instead of using the redox mediators, do the electrons transfer by other mechanism, provoking a decrease of power output. Rochex et al. (2008) concluded based on their studies that higher shear rates decreased the diversity of the biofilm, maintaining a biofilm with higher activity because of the lower maturation and keeping it young (Oliveira et al., 2013; Pinto, 2014).

For the wastewater treatment it is important to have an active and developed biofilm for continuous flow with different flow rates, so is important that the microorganisms had this capacity. The time available for the formation of the biofilm is also an important factor to have into account. This time is called the hydraulic retention time (HRT) and if it is not enough the digestion of organic compounds will not be so high and efficient (Pinto, 2014).

The studies regarding the effect of flow rate on the MFC performances showed that an increase of performance is achieved with an increase of the flow rate until a major value, after that the power output decays. It occurs because the bacteria concentration decrease and therefore the energy produced, also, decreases.

3.6 Scale up

As already mentioned, one of the main disadvantages of the MFC is the low power outputs and the high costs associated to the materials used. For use this technology in real applications, like wastewater treatment plants, it is necessary to developscale up MFC. To do that, low cost materials are required and the importance of a simple construction, systems with easy maintenance, the reactor configuration, the operation at bigger scale, the performance of the electrode and the durability are main issues. The catalysts used (as platinum) are very expensive and make large scale applications difficult. The biggest challenge is to keep the power values needed to use in real applications.

Tender et al. (2008) used for the first time a MFC as an alternative power source to a meteorological buoy that measures a set of parameters. This is a low power consuming device that works in the bottom of marine environments, it is called Benthic Microbial Fuel Cell (BMFC). In earlier studies, the scale up process demonstrated a decrease in the power achieved with an increase of the reactor size (Oliveira et al., 2013).

The scale up process could be done in two ways: increasing the volume of a single MFC or connecting a set of single small units. The volume increase can change the space between the electrodes and cause alterations in the internal resistance and create an irregular current distribution. Therefore, it is necessary to know how the size, the surface area of the electrodes and how the space between them affects the power output in scale up MFCs. Some studies was already performed, Dewan et al. (2008), discovered that the maximum potential is not directly proportional to the anode surface area, but that proportionality is related with the logarithm of the surface area. Cheng and Logan (2011) found that the power can increase 12% and 62% with the duplication of the anode and cathode size. The best option is to use three dimensional electrodes which allows an increase in the electrode surface in the same reactor volume (Oliveira et al., 2013).

The impact of the space between electrodes was studied by Liu et al. (2008). The authors determined that this parameter affects the specific area, the internal resistance and consequently the power generated. In addition, the distance between the electrodes could contribute to an irregular current distribution and affect the biofilm growth (Oliveira et al., 2013).

The ohmic losses,also, increased because the distance between the electrons generation and the final acceptor is greater. This effect was studied by Cheng et al. (2014) and they found that modifying the connecting configuration and the material of the anode, the power loss could be reduced (Oliveira et al., 2013; Logan, 2008).

Another way of doing the scale up is connecting a set of single MFC, in series or parallel. Ioropoulos et al. (2008) have done a group of tests comparing the performance of MFC with different sizes and under continuous conditions. They also carried out the connection of small units in series, parallel and both in order to determine which presents the best performance. The best results were achieved for series-parallel tests. This method seems to be the better way of do the MFC scale-up, however, this connection present some problems like unpredictable operation, voltage losses and voltage reversal. Another problem that can arise is that the best material for one MFC cannot be the better option for another cell. As an example, proton exchange membranes that has good results in a lab scale may not present the best performance for stacks (Oliveira et al., 2013).

3.7 Main goals for the present work

The main goals for the present work were already reported in the introduction chapter. At this point it is important to relate the objectives of the work with developed studies already performed by other authors. As mentioned and described, a lot of parameters affects the MFC performance, so, it is important to explore how they affect the cell behaviour. As stated some studies were developed in order to understand better how they affect the performance Different tests were performed but it is difficult to have a term of comparison since all the cases presented were performed in different conditions, nevertheless, the work already performed with MFCs can be used as a starting point towards a better understanding and development of these systems.

Having in mind the critical literature review presented above and since there is a lack of published work on MFCs operating with *Lactobacillus Pentosus,* it is an objective of this workto study the effect of configuration and operating conditions, such as operating mode, cell configuration, membrane area, anode electrode area, yeast extract concentration and membrane thickness on the MFC power output, COD removal rate and biofilm formation on the anode side.

4 Technical Description

4.1 Materials and methods

4.1.1 Synthetic residual water

As mentioned, it was used a synthetic effluent simulating a wastewater of a dairy industry as a grow environment to the bacteria used. The constitution of this synthetic residual water (SRW) is given in the Table 4.1:

Table 4.1-Composition of the synthetic wastewater(Remya et al.).

Reagent		Concentration (mg/L)
Name	**Formula**	
Glucose	$C_6H_{12}O_6$	85
Yeast extract	-	5
Milk powder	-	1300
Amido	$(C_6H_{10}O_5)_n$	5
Amonium chloride	NH_4Cl	20
Dipotassium hydrogen phosphate	K_2HPO_4	22
Potassium dihydrogen phosphate	KH_2PO_4	11
Magnesium Sulfate Heptahydrate	$MgSO_4.7H_2O$	78
Calcium carbonate	$CaCO_3$	35

4.1.2 Inoculums preparation and bacteria grow

The bacteria used was *Lactobacillus pentosus*. This microorganism was already available in the laboratory but it was necessary some preparation to add it to the synthetic effluent used. In a first stage it was necessary to grow it in a specific media by inoculation in liquid MRS (grow media) during 2 to 3 days. After that, the solution was centrifuged at 4000 rpm, during 15 minutes, provoking the separation of the liquid media and the biomass. The biomass was transferred to a flask with 300 mL of synthetic residual water and was leaving on it to grow during more 2 days. In the beginning of the experiments these 300 mL were introduced in the anode chamber and the rest was filled with the synthetic effluent.

4.1.3 Configuration of the Microbial Fuel Cell: Single and Dual Chamber

In this study two different configuration of the Microbial Fuel Cell were used: a single and a dual chamber MFC.

The dual chamber MFC is composed by two chambers with the same volume (1L). The first compartment, the anode, was filled with synthetic residual water and the bacteria. The second one, the cathode, was filled with distillate water and an air-sparger was fixed to promote the dissolution of the oxygen during the MFC operation. Both compartments were physically separated by a proton exchange membrane (PEM) (Figure 4.1).

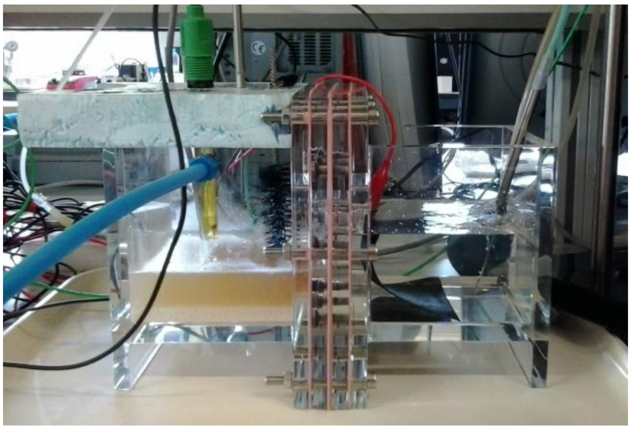


Figure 4.1- Dual chamber Microbial Fuel Cell.

The single chamber Microbial Fuel Cell is composed by only one chamber, the anode chamber. The cathode, in this case, stays side by side the membrane and is opened to the air.

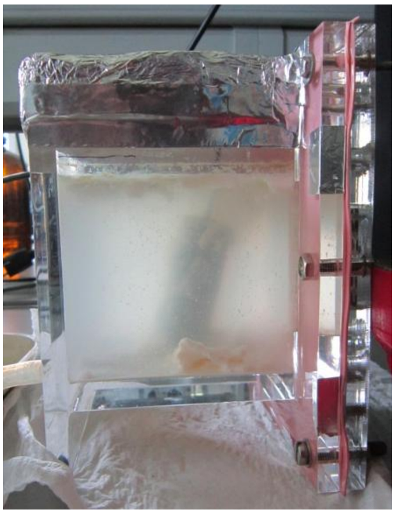


Figure 4.2 - Single chamber Microbial Fuel Cell.

In both cases the anode chamber has to be under anaerobic conditions. Based on that, the top of the compartment was cover with expanded polystyrene and isolated around to avoid the entrance of air. Two holes were left, one to grab the electrode and another to take samples. In the Batch operation this last one was used to reintroduce the substrate.

4.1.4 Microbial Fuel Cell components

In addition to the different configuration tested, it was also used two different membrane areas. In the tests with the double chamber MFC the membrane area was 25 cm^2 and 42.25 cm^2. In all the tests made with single chamber the membrane area was 25 cm^2.

The membranes used were from Nafion 212 (0.051 mm) and Nafion 117 (0.183 mm), both brought to QuinTech, a Germany Company.

In the anode chamber it was used a graphite brush (from "The Mill-Rose Company", USA) working as electrode allow the electrons collection and biofilm formation (Figure 4.2). The effect of the anode electrode on the MFC was evaluated, so two sizes of brush (one with 3 cm and other with 7.5 cm) were used.

4.3- Anode electrode (brush with filaments of carbon fibre).

On the cathode chamber a plain carbon cloth with 100 cm^2 (from “Fuel Cells Etc”) treated with 1 mg/cm^2 Platinum Black was used as cathode electrode. As mentioned, in the single chamber the cathode stays next to the membrane, in double chamber this tissue was immersed in the water.

4.1.5 Operation mode

Both types of reactors (dual and single) were operated in two different modes. In a first phase the reactors worked in a continuous mode with a permanent flow rate of 0.05 L/h controlled by a peristaltic pump. In this case the period of the experience was one month and the polarization and the others measurements (CQO) were realized once per week.

The experiences were also performed in batch mode, in cycles of 48 hours and with a total duration of 15 days. In this case the polarization curves and the others measurements were realized in periods of two days.

4.1.6 Main procedure

As was said, according with the type of configuration of the MFC or the operation mode the tests were realized along one month or fifteen days. It was established a main procedure to realize it in all the cases and in each day that was done the polarization measurements to maintain the same conditions for each test.

The polarization curves were performed in a electrochemical work station (Zahner - Electric GmbH & CO) (Figure 4.4) and the test were performed in galvanostatic mode (set the current and measure the cell voltage).

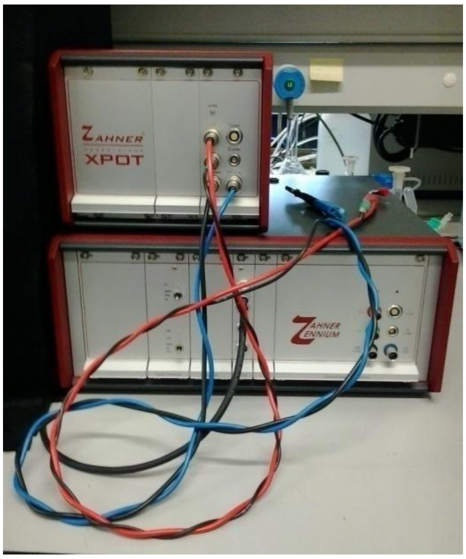


Figure 4.4-Zahner - Electrochemical station.

The open circuit conditions were maintained during 15 minutes to stabilize the cell. After that, and every 3 minutes, the current was increased with a constant increment of 0.01 A and the cell voltage was registered. With these values it was possible to do the polarization curve, verifying the behaviour of the voltage (V) as a function of the current (mA) and consequently the behaviour of the power density (mW/m^2) as function of the current density (mA/m^2).

As explained the Power is a product of voltage (U) and current (I). Usually the current is presented by the membrane area (m^2). Once the amount of energy produced and consequently the cell performance is affected by that.

$$P = U \times I \tag{5}$$

And with the normalization the power density is obtained by:

$$P = U \times \frac{I}{A} \tag{6}$$

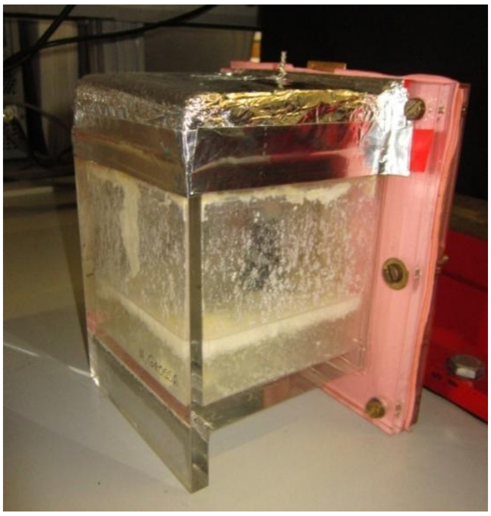
After this test samples from the reactors to accomplish microbial platings (appendix A) were collected, allowing the counting of colony forming units (CFU). Was used a selective medium (MRS) for the bacteria growth and allowing the isolation of the *Lactobacillus*. This medium was prepared based on the indications of deMan, Rogosa and Sharpe (Neogen corporation, 2010).

Were also analysed the chemical oxygen demand - COD (appendix B) to know the amount of organic compounds in the samples based on the quantity of oxygen used to oxidize the organic matter (Logan, 2008). This test was performed using the potassium dichromate reflux method. These tests were effectuated to the effluent inside the anode chamber before the polarization curve measurements.

The COD removal was estimated following the equation:

$$COD_{removal} = \frac{COD_{SRW} - COD_{effluent}}{COD_{SRW}} \tag{7}$$

In the batch mode operation, after this procedure 2/3 of the anode chamber solution was removed and replaced with new solution (SRW).



4.5- Single chamber Microbial Fuel Cell with 1/3 of the substrate.

To each samples it was also analysed the pH, temperature and the sugars quantification (appendix C). After the experience time (one month of fifteen days) it was performed the biofilm extraction (appendix D) that was formed in the filaments of the anode electrode.

Figure 4.6 - Biofilm formed in the brush of carbon fibre - anode.

This biofilm was characterized by the quantification of sugar and by the quantification of proteins (appendix E) follow by the Debois Method.

5 Results and Discussion

As previously mentioned, the main goal of this work was to study the effect of different configurations of a Microbial Fuel Cell on its overall performance (energy production, wastewater treatment and biofilm formation). In order to achieve that, different tests were carried on the design MFC. Experiments were performed to study the best operation mode, continuous versus batch and for each operating mode various configurations were tested. The different tests included changes in membrane area, in the anode electrode size, in the yeast amount and in the membrane type.

As stated, the Microbial Fuel Cell performance was evaluated based on a set of parameters that can be analysed by the polarization curves, the COD removal values, the biofilm characterization and other parameters that were monitored throughout each experiment. The different tests performed and their characteristics are shown in Table 5.1.Due to the large amount of tests performed and results obtained, in each subsection of this chapter a sub-set of conditions was selected and is presented. The remaining results can be found in Appendix F and Appendix G.

Table 5.1 – Operating conditions.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
1	Dual	Continuous	BP 1"	25 cm^2	Nafion 212	5
2	Dual	Continuous	BP 3/4"	25 cm^2	Nafion 212	5
3	Dual	Continuous	BP 1"	42,25 cm^2	Nafion 212	5
4	Dual	Continuous	BP 3/4"	42,25 cm^2	Nafion 212	5
5	Single	Continuous	BP 1"	25 cm^2	Nafion 212	5
6	Single	Continuous	BP 3/4"	25 cm^2	Nafion 212	5
7	Single	Batch	BP 1"	25 cm^2	Nafion 212	5
8	Single	Batch	BP 1"	25 cm^2	Nafion 117	5
9	Single	Batch	BP 1"	25 cm^2	Nafion 212	50
10	Single	Batch	BP 1"	25 cm^2	Nafion 117	50

5.1 Effect of the operation mode

As mentioned the tests performed with the design MFC were performed in two different operation modes: continuous mode and batch mode. These tests were carried out to analyse the effect of the operating mode on the MFC performance. In Table 5.2 the characteristics of both experiments are displayed.

Table 5.2 – Experiments performed to compare the operation mode.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
5	Single	**Continuous**	BP 1"	25 cm^2	Nafion 212	5
7	Single	**Batch**	BP 1"	25 cm^2	Nafion 212	5

The results obtained are shown in Figure 5.1, where two of the polarization curves for the different reactors are presented. For the MFC in the continuous mode, the 2nd and 3rd polarization curves were selected to be presented here, for the batch mode the 2nd and the 6th polarization curves were choosed.

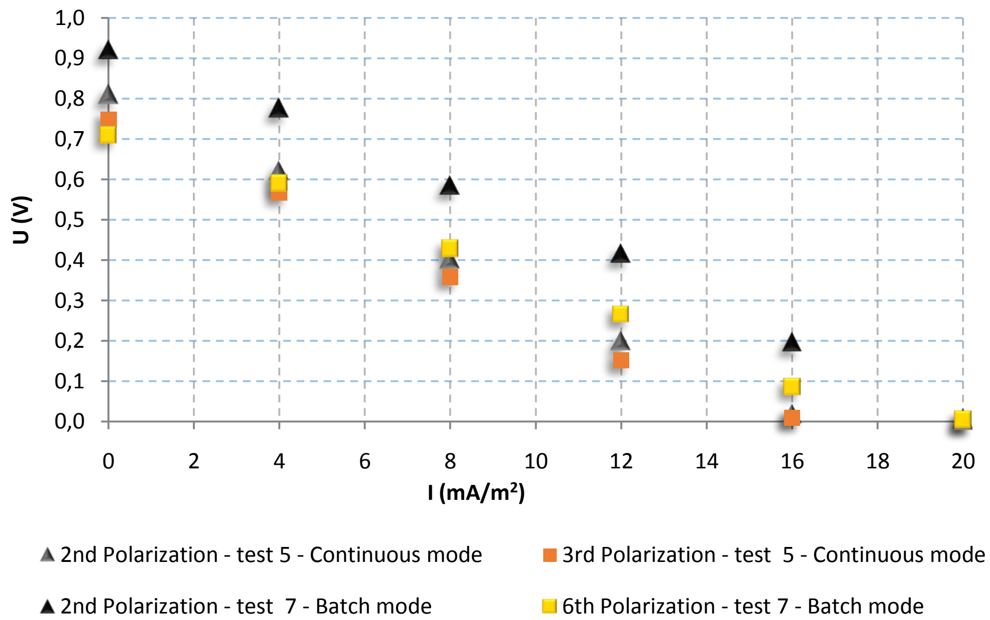


Figure 5.1- Performance of a microbial fuel cell for different operation modes.

By the analysis of the polarization curves, it is evident that for the continuous mode, the 2nd polarization shows a better performance than the 3rd, since the voltage values, for the same current density, are higher. This corresponds to higher power densities (Table 5.3). As was explained before, the polarization curves can be divided in three different voltage losses regions. The first one, for lower current densities, is dominated by activation losses. The second one, with medium values of I, is controlled by ohmic losses. In the third, potential losses usually called concentration losses are due to mass transfer limitations and occur for higher current densities. In the plots, a little difference between both polarizations occurs at low and medium current densities. The differences at low current densities could be explained by a higher loss of potential due to the transport of the different species to the anode surface. This occurs because in the third polarization the amount of biofilm in the anode electrode is higher, creating a higher resistance to mass transport from the anode media to the electrode surface, where the reactions take place and where the electrons are collected. Also, at the middle of the operating range, lower voltage values are obtained for the third polarization. In this range the major lossis the ohmic loss,which is due to the transport of electrons and protons in the cell. Since in the third polarization the biofilm attached to the anode electrode has a higher thickness, it difficults not only the transport of the different reactant species, but also increases the resistance to the transport of electrons to the electrode and the protons to the membrane. In the concentration region, the voltage drop occurs more abruptly in the 3rd polarization than in the 2nd. This can be explained by the fact that in these conditions the microbial activity could be decreasing, so the rate of substrate oxidation also decreases. Due to a higher biofilm thickness on the anode electrode, the amount of substrate that reaches the electrode decreases with a consequent decrease on the oxidation rate.

For the reactor operating in batch mode the cell behaviour represented by the polarization curves is quite similar, with exception on the 3rd region, since in this case, the voltage drop is a little higher for the 2nd polarization. In the case of the batch mode the voltage values are substantially higher for the 2nd polarization. Like in the continuous mode, the main explanation for that, is the increase of the biofilm thickness in the anode electrode and in the membrane. This will increase the mass transport resistances of the different species with a consequent decrease of the cell performance.

Comparing the two operation modes it is possible to verify, in a first analysis, that the best performance is achieved in batch mode (Figure 5.1 and Table 5.3). Comparing the voltage losses it is difficult to identify big differences, but in general the losses of the assay 7 are less noticed. Only in the 3rd polarization of the assay 5 (continuous mode) was felt that for higher current densities the performance of the cell decreases significantly. This could be explain by the fact that at this moment the reactor is operating at three weeks and the microbial activity could be decreasing due to contamination or reduction of the amount of bacteria in the anode media. Also, the continuous flow of the synthetic wastewater, in the continuous mode, washes out the microbial community, decreasing the microbial concentration in the reaction media and consequently the energy production.These severe conditions also affect the biofilm formation, maturity and stabilization leading to thinner biofilms attached to the electrode surface (Table 5.4) and consequently lowers power densities.

It should be mentioned that the hydraulic retention time (HRT) for the two modes of operation is different being higher for the batch mode. In order to achieve the best performance with a MFC, the time needed for the formation of a biofilm is other important parameter that should be considered. Thus the HRT is an important parameter that needs to be addressed for the optimization of a MFC. Is seems that an optimal performance is obtained after microbial community has been given time to develop and when nutrient capture and the extent of hydrolysis of substrate are more favourable. Also, a decrease of the HRT leads to a decrease of the power output and COD removal, due to a decrease of the time available for the microbial community digest the organic compounds and to the biofilm formation (Table 5.3 and Table 5.4).

The MFC power densities and the COD removal values are summarized in Table 5.3:

Table 5.3 – Power densities and COD removal values for each polarization curve.

	Test 5		Test 7	
Polarization nr.	P (mW/m^2)	COD Removal (%)	P (mW/m^2)	COD Removal (%)
1	5.36	62	4.46	54
2	3.23	66	5.00	66
3	2.87	58	4.41	66
4	2.70	66	3.29	60
5	-	-	3.19	54
6	-	-	3.42	56
7	-	-	3.30	48

In the continuous mode the higher power densities obtained in the 1^{st} (5.36 mW/m^2) and 2^{nd} polarization, following that order. In the Batch mode the best power density values were reached in the 2^{nd} polarization (5.00 mW/m^2), followed by the first one. Regarding the COD removal rate, in both cases it is possible to reach values always above 50%. In the continuous mode the best power density does not correspond to the best COD removal, since this was reached in the 2^{nd} polarization (66%). In the batch mode the best COD removal (66%) and power density values were achieved in the same polarization (2^{nd} one).

As already mentioned, the biofilm formed in the anode has a preponderant role on the MFC performance since it affects the electrons conduction and the substrate degradation. It could also give us information about the amount of bacteria that is attached to the anode electrode. Based on that, it is extremely important to quantify and characterize the biofilm formed in each experiment. The values obtained for the different operating modes tested are presented in Table 5.4.

Table 5.4 – Biofilm characterization - quantification of sugars, proteins, dry weight and CFUs.

Test	Sugar (mg/mg VSS)	Protein (μg/mg VSS)	Dry weight (mg/mL)	Colonies (CFU/mL)	α (CFU/mg)
5	0.132	302.2	1.60	2.10E+07	1.32E+07
7	0.006	366.7	3.23	2.63E+08	8.14E+07

In test 7, the amount of sugar is lower than in test 5. This may indicate the existence of a better degradation of the substrate and a better electrons transfer in this case. These results are in agreement with the higher performances and power outputs shown before.

The value of the protein and dry weight is bigger for the MFC operating in the batch mode. In this case, the reactor does not have a continuous feed of substrate and therefore the bacteria have more time to adapt to the anode media and to adhere to the electrode surface. Higher biofilm thicknesses present some disadvantages such as the increase of losses due to mass transfer limitations and consequently the MFC performance decreases. However, it is positive to achieve an “optimal” biofilm thickness to promote the activity of the bacteria and the electrons transfer from the microorganism cells to the electrode surface.

In the beginning of each experiment a set of CFU was prepared and was added to the SRW. However, ensuring an equal quantity of that in each experiment is very difficult and not possible. Based on that the variable, α, was introduced to normalize that. This variable is related with the dry weight of the biofilm and the amount of bacteria presented in the beginning of each test. The dry weight, as mentioned, has a higher value for the batch mode, but the number of colonies in the beginning of the test was also higher. Based on the results, it is possible to say that the amount of bacteria in the biofilm formed in test 7 was higher than in experiment 5. This is in accordance with the results presented above and show that the batch mode conducted to a better overall performance of the MFC tested in this work.

For a real application it is necessary to have into account that batch mode is not so appropriate for power production and wastewater treatment. The continuous mode has some advantages and has been more developed. The growth kinetics and kinetic constants are determined with higher precision, and it was found that it is possible to adjust the medium to achieve the maximum productivity (Rahimnejad et al., 2011).

5.2 Continuous reactor

After the comparison between the two operation modes, different conditions were tested in each configuration in order to increase the MFC performance. For continuous mode, the effect of the cell configuration, the membrane area and the area of the anode electrode on the MFC performance was studied. The results obtained are exposed in the following subchapters.

5.2.1 Effect of the cell configuration

To study the effect of the cell configuration of the MFC performance two different configurations were tested: dual chamber and single chamber. In Table 5.5 are exposure the characteristics of each experiment performed are presented.

Table 5.5– Operating conditions used to evaluate the effect of the cell configuration.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
1	**Dual**	Continuous	BP 1"	25 cm^2	Nafion 212	5
5	**Single**	Continuous	BP 1"	25 cm^2	Nafion 212	5

The methodology followed for these experiments was the same as for the others and as can be seen in Figure 5.2 the polarization curves presented are for the 2^{nd} and 3^{rd} polarization.

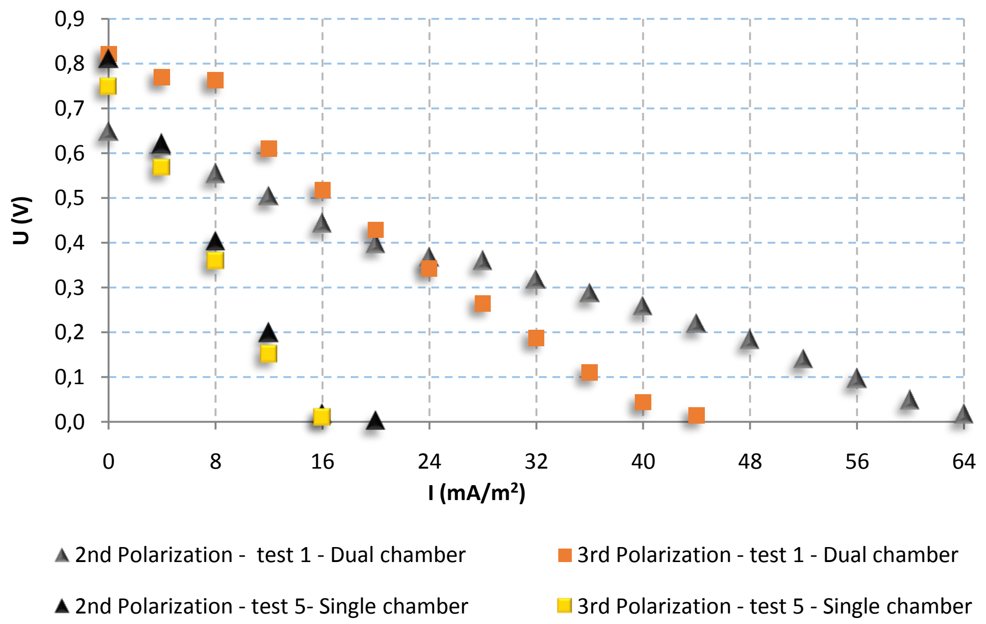


Figure 5.2- Performances of the MFC with different cell configurations.

Based on the results presented, it is possible to note that the polarizations curves had a different development as the current density increases. For both configurations, the slope of the 3rd polarization curves is higher than the 2nd meaning that the losses occur in a more pronounced way. Having in mind the three losses regions, we are able to verify that in the 2nd polarization curve of the cell with a single chamber design, the voltage values reached are not so high when compared with the 3rd polarization. For the dual chamber design, it is verified a fall of U at low current densities, but for the 2ndpolarization this fall is much higher which means that the activation losses were more pronounced in this case. The values obtained in the open circuit are much higher in the 3rd polarization,but in the 2nd one, the cell stays more constant and reaches higher values of current density. For medium current densities, the behaviour is similar, with losses more significant in the 3rd polarization.The main losses in this experiment are felt in this intermediary phase. With the passage of time, the biomass accumulation in the different parts of the cell increases gradually. This accumulation increases the resistance and the passage of electrons and protons is harder and the voltage values decrease. In both polarizations, it is possible to see a decrease of voltage values for high values of current densities caused by some limitation in the concentration of reactants.

Comparing the two configurations it is possible to notice that, the best performance was achieved in a dual camber MFC. Although a dual chamber has higher transport resistances due to a higher distance between the electrodes it, also, has oxygen injection/bubbling which benefits the cathode reaction and can maintain two different pH conditions to optimize the anodic and cathodic reactions. In a MFC,the anode reaction produces protons and the cathode reaction consumes protons. Accumulation of protons due to slow and incomplete proton diffusion and migration through the membrane will cause a pH decrease in the anode. This will lead to a decrease of bacterial activity on the anode side and consequently will affect the electron transfer at this side. Therefore, most MFCs operate at neutral pH in the anodic compartment in order to optimize bacterial growth conditions. The continuous proton consumption by the oxygen reduction reaction results in a pH increase at the cathode side which according to the Nernst equation results in a decrease in current generation since the potential of the oxygen reduction reaction should decrease with an increase of the pH value. Therefore, to achieve higher power outputs, it is needed to have neutral pH at the anode side and lower pH values at the cathode side.A dual-chamber MFC can maintain two different pH conditions to optimize the anodic and cathodic reactions. However, this is impossible to do in a single chamber MFCs, because only one chamber is present.

The results of the biofilm quantification, also show that the single chamber configuration leads to thicker and dense biofilms. In this case, the mass transport resistance of the different reactant species and electrons from the anode media to the electrode surface will be higher, adversely affecting the cell performance.

The power densities and COD removal values for each configuration and polarization curve are presented in Table 5.6.

Table 5.6 - Power densities and COD removal values for each configuration tested.

	Test 1		Test 5	
Polarization nr.	P (mW/m^2)	COD Removal (%)	P (mW/m^2)	COD Removal (%)
1	6.62	12	5.36	62
2	10.37	46	3.23	66
3	8.58	45	2.87	58
4	6.78	73	2.70	66

Based on the results achieved, it is possible to conclude that the experiment carried out with a double chamber presents a better performance, since the values obtained for power density are all higher than for the assay performed in a single chamber. The maximum power output achieved in the dual chamber MFC was 10.7 $mW/m^2$and with the single chamber was 5.36 mW/m^2.Taking into account the COD removal the analysis is quite different. In this case it is possible to verify that in all the measurements performed, the COD removal for test 5 is higher than 58% and higher than the values of experiment 1. In the dual chamber configuration, only one value is greater than 50% and this value is reached in the last week. In this case the major value of power density corresponds to the best COD removal. These results suggest that the single chamber configuration allows better substrate degradation, but the electrons produced were not conveniently transformed in electricity production.

The values for the different parameters used to characterize the biofilm are exposure in the Table 5.7.

Table 5.7 - Biofilm characterization - quantification of sugars, proteins, dry weight and CFUs.

Test	Sugar (mg/mg VSS)	Protein (μg/mg VSS)	Dry weight (mg/mL)	Colonies (CFU/mL)	α (CFU/mg)
1	0.074	329.9	0.98	5.00E+06	5.12E+06
5	0.132	302.2	1.60	2.10E+07	1.32E+07

Based on the results presented regarding the effect of the MFC design on the MFC performance, it could be concluded that the experiment performed with the dual chamber presents a better performance if we have into account, only the power density achieved. However, the single chamber cell was able to reach greater values for COD removal being more efficient for effluent treatment. A single chamber MFCs configuration is more advantageous for practical applications due to its simplified reactor configuration, therefore, it is desirable to find a way to work with this configuration maintaining a higher power output.

5.2.2 Effect of the membrane area

In order to evaluate the effect of the membrane area on the MFC performance, experiments using two different membrane areas: 25 cm^2 and 42.25 cm^2 were performed. The characteristics of the cells used to perform these tests are presented in Table 5.8.

Table 5.8 - Tests performed to evaluate the effect of the membrane area.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
2	Dual	Continuous	BP 3/4"	**25 cm^2**	Nafion 212	5
4	Dual	Continuous	BP 3/4"	**42,25 cm^2**	Nafion 212	5

The corresponding polarization curves are presented in Figure 5.3 and the power density and COD removal values are shown in Table 5.9.

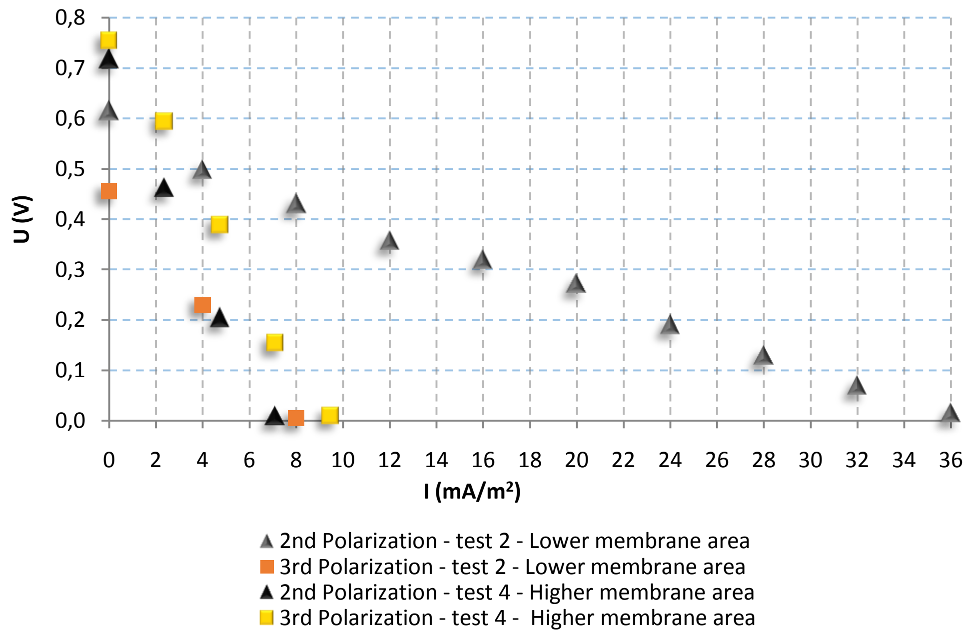


Figure 5.3- Performances of the MFC with different membrane areas.

Regarding the cell with the lower membrane area, as can be seen, the performance of the 3rd polarization curve is much lower than the 2nd. The slope of the curve for the 3rdpolarization is very high which means that the potential of the cell decreased abruptly. For the 2nd polarization the behaviour is more linear with progressive decrease of voltage for higher values of current density. For the cell with the higher membrane area, the results are opposite. In this case, it is possible to notice that the performance of the cell is better in the 3rd polarization.

Comparing the curves of both experiments it can be seen that for low current densities a higher membrane area generates better results. This is due to the fact that a higher area allows more protons transfer from the anode to the cathode side increasing the oxygen reduction reaction rate and reducing both the activation and the ohmic losses. Contrarily, for higher current densities a better performance was achieved with the MFC with lower membrane area. This may be explained by an accumulation of protons on the cathode side due to their higher transfer rate through the membrane which will lead to a pH increase on the cathodic compartment and a decrease in current generation since the potential of the oxygen reduction reaction decreases with an increase of the pH value. Also, the biofilm formed on the MFC with a higher membrane area is thicker, dense and more active, since it has a higher bacteria concentration per biofilm weight (Table 5.10) allowing a higher electron transfer rate, which contributes especially for reducing the activation and the ohmic losses. This higher thickness only is disadvantageous for high current conditions, since in this region the major loss is the concentration loss which is due to mass transport limitations. An increased biofilm will lead to an increased mass transport resistance of the different species to the electrode surface decreasing the substrate degradation rate, as can be seen by the COD removal values in Table 5.9.

Table 5.9 - Power densities and COD removal values for different membrane areas.

	Test 2		Test 4	
Polarization nr.	P (mW/m^2)	COD Removal (%)	P (mW/m^2)	COD Removal (%)
1	0.06	62	1.09	61
2	5.44	16	1.10	54
3	0.924	67	1.84	61
4	2.00	2	0.18	74

The best power density was reached in the assay 2, 5.44 mW/m^2, and in the assay 4 the best one was of 4.73 mW/m^2. The major value for the COD removal,was obtained in the cell with higher membrane are and was 74%. For the assay 2 the best COD removal was 67%.

The parameters regarding the biofilm characterization for the performed experiments to evaluate the MFC performance with different membrane areas are present in Table 5.10.

Table 5.10 - Biofilm characterization - quantification of sugars, proteins, dry weight and CFUs.

Test	Sugar (mg/mg VSS)	Protein (μg/mg VSS)	Dry weight (mg/mL)	Colonies (CFU/mL)	α (CFU/mg)
2	0.100	223.8	1.62	1.83E+08	1.13E+08
4	0.052	223.9	0.71	1.47E+07	2.07E+07

The sugar values are consistent with the values obtained for the COD removal since experiment 4 presents a lower value. This represents a better degradation of the substrate by the bacteria, which leads to higher values for the COD removal.

The value for the proteins is identical in both conditions and since the biofilm dry weight was lower in experiment 4, the biofilm thickness was also lower. This could be beneficial because it decreases the mass transport resistances in the anode electrode, but also, leads to a less active biofilm with a lower amount of active bacteria. If we analyse the parameter α, for the same amount of biofilm more bacteria are present in the biofilm formed in test 2. In this experiment, a more active biofilm attached to the electrode surface is generated, degrading more substrate and producing more electrons and consequently more power (Table 5.9).

In summary, none of the experiments produced an overall best performance, since the cell with the lower membrane area achieved better power densities but lower COD removal values. An advantage of using a membrane with a lower area is the costs reduction.

5.2.3 Effect of the anode electrode area

As mentioned in the technical description, a graphite brush was used as anode electrode and since the biofilm attaches to the brush it is very important to study the effect of its area on the MFC performance. Therefore, two different electrode sizes (bigger BP 1" and smaller BP 3/4") were used to evaluate the effect of the anode electrode area on the MFC performance(see Table 5.11).

Table 5.11 - Experiments performed to evaluate the effect of the anode electrode area.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
3	Dual	Continuous	**BP 1"**	42,25 cm^2	Nafion 212	5
4	Dual	Continuous	**BP 3/4"**	42,25 cm^2	Nafion 212	5

The polarization curves for the dual chamber MFC are presented in Figure 5.4, the power densities and COD removal values can be found in Table 5.12 and the results obtained from the biofilm analysis in Table 5.13.

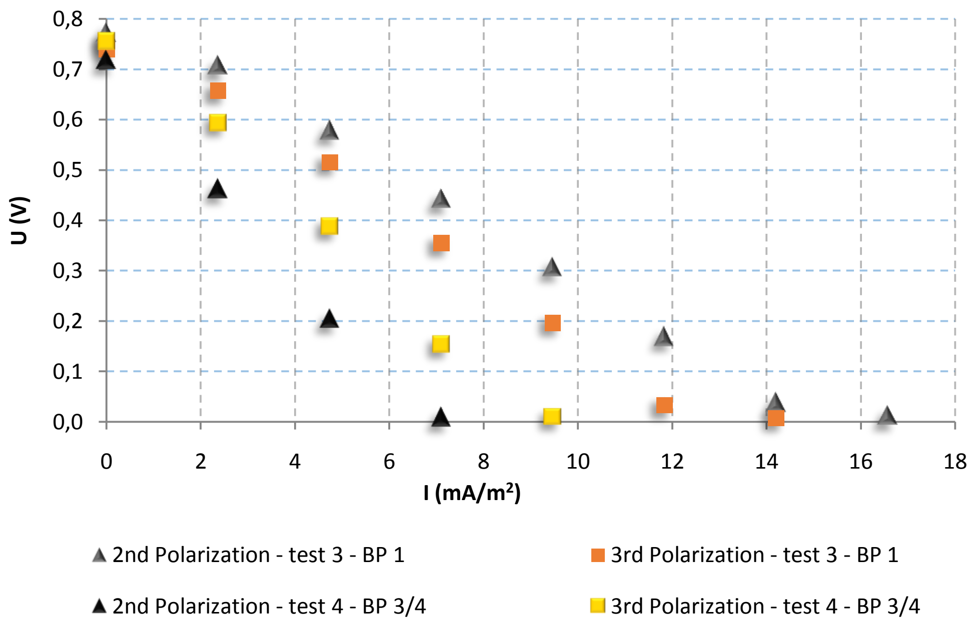


Figure 5.4- Performances of MFC with different anode sizes.

As can be seen in Figure 5.4, for the bigger electrode a higher performance was achieved in the second week and for the lower one the best performance was achieved in the third week.This may be due to the fact that for the bigger electrode the biofilm growth between the 2^{nd} and 3^{rd} polarization curves creates an additional resistance to electrons transport and collection, decreasing the cell performance. Despite that, the fuel cell performance and power output was increased with an increase in the electrode area (Table 5.12). It should be here mentioned that the electrode area is one of the important parameters that affect the MFC performance, since it is directly related to the electrons transfer and to the bioelectrochemical reactions occurring in the anode compartment. If the electrode has a lower area, it has a lower specific surface area and a consequent lower electrons transfer rate and less bacteria attached to their surface. This will lead to thinner and less active biofilms (Table 5.13) and lower fuel cell performances. However, as can be seen in Table 5.12, lower electrodes have higher COD removal values, meaning that the majority of the organic matter present in the effluent was used by bacteria but do not contribute to electricity generation. This is due to the inability of the electrode to collect all the electrons produced because of a decreased electrode surface area.

Table 5.12 - Power densities and COD removal values for different anode electrode sizes.

	Test 3		Test 4	
Polarization nr.	P (mW/m^2)	COD Removal (%)	P (mW/m^2)	COD Removal (%)
1	2.37	55	1.09	61
2	3.15	-75	1.10	54
3	2.52	70	1.84	61
4	2.29	57	0.18	74

As already mentioned and as can be seen in the Table 5.12, it is possible to conclude that the Experiment 3 had a better performance since the power density for each polarization is greater than all the power densities achieved by test 4. Once again these results support the explanation that a bigger brush electrode promotes a higher concentration of bacteria in the electrode and a higher electron transfer rate. In this case, the maximum power density achieved was 3.15 mW/m^2. Regarding the COD value of the 2nd polarization of test 3, it should be mentioned, that in this test a big amount of biomass was stuck in the tube and the sample used to measure the COD value had an excessive amount of organic matter which triggered this error. Despite that, higher COD removal rates were achieved with the lower electrode, with a maximum rate of 74%.

Table 5.13 - Biofilm characterization - quantification of sugars, proteins, dry weight and CFUs.

Test	Sugar (mg/mg VSS)	Protein (µg/mg VSS)	Dry weight (mg/mL)	Colonies (CFU/mL)	α (CFU/mg)
3	0.228	766.4	1.09	2.40E+08	2.19E+08
4	0.052	223.9	0.71	1.47E+07	2.07E+07

All the parameters presented in table 5.13, support the fact that in experiment 3, the biofilm formed was thicker, more dense, since the amount of proteins and sugar is higher. In this case the biofilm is also more active, having a higher amount of bacteria per biofilm weight. The sugar values reveal that better substrate degradation occurs in experiment 4. These results are in accordance to the COD removal values presented in Table 5.12.

In summary, it was possible to verify that a higher anode electrode leads to a higher energy production but worse wastewater treatment. Also, bigger electrodes have higher costs since they require a higher amount of carbon fibbers and other materials.

5.3 Batch reactor

As mentioned, two different operating modes were studied in this work. In this subsection,the results obtained with the batch mode are presented and show the effect of the amount of yeast extract and the membrane thickness on the MFC performance (polarization and power output), COD removal and biofilm characteristics. These effects will be discussed in detailed below. Due to a large amount of results the polarization curves that will be presented in this subchapter are for the 4nd and 8th days of operation, 2nd and 6th polarization, respectively. However, all the data can be found in Appendix G.

5.3.1 Effect of the amount of yeast extract

All the results presented were obtained using the same value amount of yeast extract as shown in Table 4.1. It is important to study the impact of the amount of yeast because itcan work as a natural mediator affecting the electrons transfer rate and consequently the MFC performance. Also, the yeast amount can work as an additional supplement to bacteria grow affecting, in this case, the bacteria activity and consequently the MFC performance. To study the effect of the yeast extract amount on a single chamber MFC two different experiments were performed, one with a the basic amount of yeast extract (5 mg/L), and the other with an amount 10 times higher (50 mg/L) ,as can be seen in the Table 5.14.

Table 5.14- Experiments performed to evaluate the effect of the amount of yeast extract.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
7	Single	Batch	BP 1"	25 cm^2	Nafion 212	**5**
9	Single	Batch	BP 1"	25 cm^2	Nafion 212	**50**

The polarization curves for this study can be found in Figure 5.5 and the power outputs and COD removal values in Table 5.15.

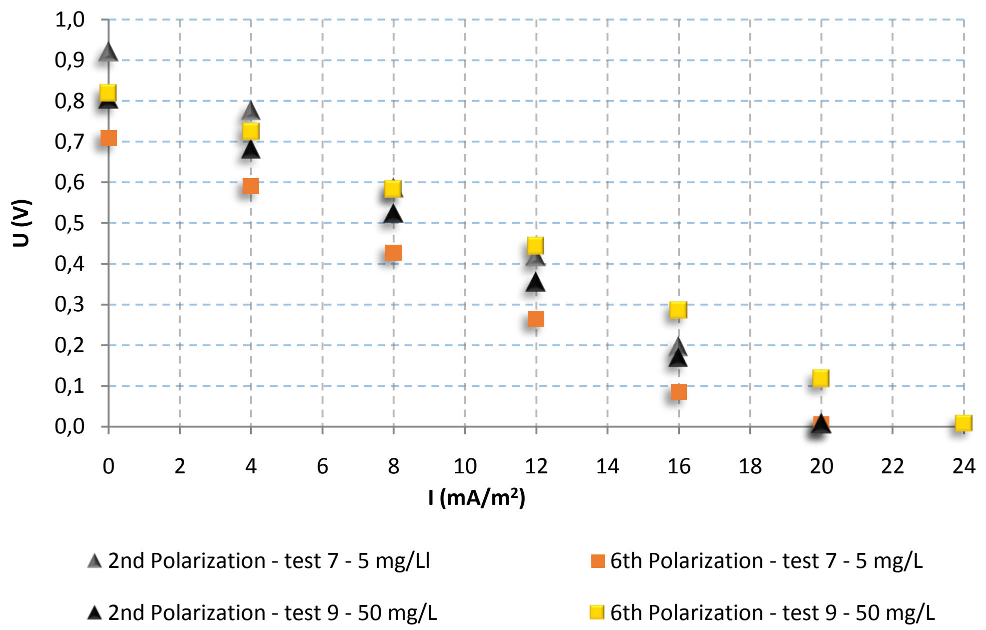


Figure 5.5- Performances of the MFC with different amount of yeast extract.

Figure 5.5 shows that in experiment 7, the performance of the cell decreases all over the time, whereas for experiment 9 the opposite occurs. This can be explained by the fact that the yeast extract acts as an additional supplement to bacteria growth avoiding some substrate depletion that occurs when a MFC is operated in batch mode. In these conditions the bacteria stays active more time, increasing their metabolic activity and consequently the fuel cell performance.

Comparing the two experiments it is possible to see that despite the major voltage value was reached for experiment 7, the performance of the cell with the lower amount of yeast extract decreases during operation and the power outputs of experiment 9 for medium and higher current densities are always higher (Table 5.15). As mentioned before, the yeast extract, can have two different effects on MFC performance. It can be a natural mediator or an extra supplement to bacteria growth. By the results presented in Figure 5.5, Table 5.15 and Table 5.16, it can be concluded that the yeast extract acts more efficiently as a mediator allowing a higher rate of electrons transfer and not as an microbial activator. This is further supported by the fact that the biofilm formed in this experiment was thicker but less active presenting a lower amount of microorganisms per biofilm weight. Therefore, the higher power outputs can be explained by the increased electron transfer rate on thicker biofilms and also by the increased electron transfer rate by the mediator.

Table 5.15 -Power densities and COD removal obtained for different amount of yeast extract with a Nafion 112 membrane.

	Test 7		Test 9	
Polarization nr.	P (mW/m^2)	COD Removal (%)	P (mW/m^2)	COD Removal (%)
1	4.46	54	4.06	57
2	5.00	66	4.26	64
3	4.41	66	4.70	47
4	3.29	60	5.03	62
5	3.19	54	5.53	52
6	3.42	56	5.32	68
7	3.30	48	4.61	66

With the values presented in the table we conclude that in test 9, with 10 times higher yeast extract amount, higher power densities values are achieved in the 5th polarization. The major power density reached was 5.53 mW/m^2 for the assay 9, and 5.00 mW/m^2 for the assay 7.

In terms of COD removal the values are not much different, with a maximum COD removal of 66% for assay 7 in the 2nd and 3rd polarizations and 68% for the assay 9 in the 6th polarization.

Table 5.16 - Biofilm characterization - quantification of sugars, proteins, dry weight and CFUs.

Test	Sugar (mg/mg VSS)	Protein (µg/mg VSS)	Dry weight (mg/mL)	Colonies (CFU/mL)	α (CFU/mg)
7	0.122	366.7	3.23	2.63E+08	8.14E+07
9	0.098	128.1	4.89	6.33E+05	1.30E+05

A question to raise is if the amount of yeast extract increases the amount of bacteria present in the anode chamber and affecting the substrate degradation and/or it acts as a mediator, increasing the electron transport rate to the anode electrode. By the analysis of the sugar in the biofilm, presented in Table 5.16, it is possible to conclude a better degradation of substrate is obtained in test 9. At the same time, comparing the other parameters it is possible to see that the colonies per biofilm weight are higher for test 7 which eliminates the hypothesis of a higher grow rate of bacteria motivated by the higher amount of yeast extract. The number of CFU/mL was monitored during all the polarization curves performed and it was possible to verify that in the beginning of test 9, the amount was 2.58E+07 CFU/mL and in test 7 was 1.74E+07 CFU/mL. However, in the last measurement performed, the results were quite different and test 7 presented a higher amount of *Lactobacillus pentosus*. This fact supports that the yeast extract increase the electrons transfer rate and not the bacteria growth on the anode compartment.

Based on the results presented, it can be concluded that a better performance was achieved with a large amount of yeast extract.

5.3.2 Effect of the membrane thickness

In a MFC, electrons migrate to the cathode depending on the potential gradient and protons are transferred to the cathode by diffusion. Since the proton transfer is slower than the electron transfer, the first one is the rate limiting step and a major cause of internal resistance. In this way, proton transfer affects the MFC internal resistance and the ohmic losses and influences the power output of the MFC.In order to create a potential gradient between the anode and the cathode side, a membrane is introduced, in a MFC, to separate each side, although the membrane also acts as a proton transfer barrier. Therefore, the membranes used are one of the most importantcomponents in MFCs, as they physically separate the anode andcathode compartments while allowing protons to pass through towards the cathode. Based on that, in this work, the effect of the membrane thickness on the fuel cell performance was studied. Two membranes with different thicknesses were used, Nafion 117 (0.183 mm) and Nafion 212 (0.051 mm) as displayed in Table 5.17.

Table 5.17- Experiments performed to evaluate the effect of the membrane thickness.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
7	Single	Batch	BP 1"	25 cm^2	**Nafion 212**	5
8	Single	Batch	BP 1"	25 cm^2	**Nafion 117**	5

The polarization curves regarding the effect of the membrane thickness on the MFC performance, the power outputs and COD removal values, as well as, the biofilm quantification are presented, respectively, in Figure 5.6, Table 5.18 and Table 5.19.

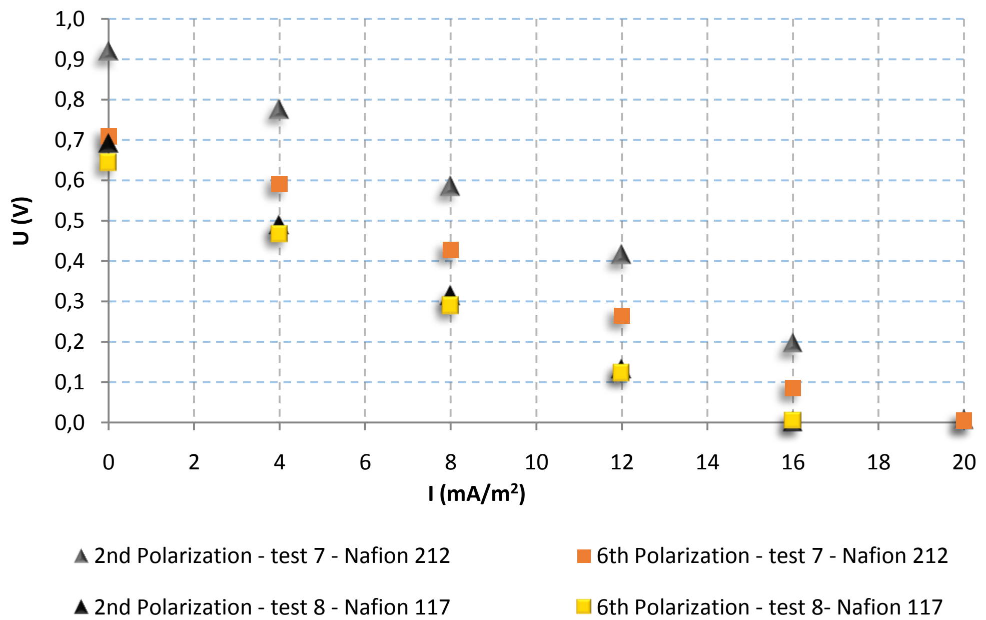


Figure 5.6 - Performances of the MFC with different membrane thicknesses.

It is evident from the plots of Figure 5.6, that, for the thinner membrane (Nafion 212) tested, the performances reached in the 2nd polarization are much higher than the values obtained in the 6thone. For low current densities this difference is higher but as the current density increases, the difference becomes less pronounced. Regarding the Nafion 117 membrane, as can be seen, the results for the two polarizations are quite similar. Although, for low current densities the 2nd polarization show a little bite higher values.

Comparing the performance of the MFC with the different membrane thicknesses, it is possible to see that test 7, with a thinner membrane, presents the best performance due to the fact that thinner membranes induce a lower resistance to protons transfer through the membrane from the anode to the cathode. This leads to a higher concentration of protons on the cathode side increasing the oxygen reduction reaction rate and to a decrease on the ohmic resistance losses on the cell due to protons transport limitations. Consequently, the performance and the power outputs reached by the cell, with the thinner membranes, are higher (Figure 5.6 and Table 5.18). It should also be mentioned that if thicker membranes limit the proton transfer rate through the membrane, a proton accumulation on the anode side occurs inducing an increase of pH on the anode compartment. The anodic pH microenvironment is one of the important factors, influencing bacteria activity and substrate degradation and in turn affecting the electron and proton generation mechanism. Generally, bacteria respond to the changes in internal and external pH by adjusting their activity. Depending on the bacteria and growth conditions, changes in pH can cause alterations in several primary physiological parameters, such as, ions concentration, membrane potential, proton-motive force and biofilm formation. Most MFCs are operated at neutral pH in order to optimize bacterial growth conditions. Therefore, the increase of pH in the anodic compartment with the thicker membrane will lead to bacteria less active with a consequent decrease of the substrate degradation rate, as can be seen by the COD removal values and power outputs, presented in Table 5.18. This will also, affect negatively the biofilm formation and the biofilm activity (Table 5.19).

Table 5.18 - Power densities and COD removal values for different membrane thicknesses.

	Test 7		Test 8	
Polarization nr.	P (mW/m^2)	COD Removal (%)	P (mW/m^2)	COD Removal (%)
1	4.46	54	2.70	40
2	5.00	66	2.52	59
3	4.41	66	2.51	52
4	3.29	60	2.51	38
5	3.19	54	2.38	49
6	3.42	56	2.31	25
7	3.30	48	2.36	16

According to the results presented in Table 5.18, the best power density was achieved with the thinner membrane, 5.00 mW/m^2, and the COD removal value was 66%. For the thicker membrane the maximum power output was 2.70 mW/m^2 and the COD removal 59%.

Table 5.19- Biofilm characterization - quantification of sugars, proteins, dry weight and CFUs.

Test	Sugar (mg/mg VSS)	Protein (µg/mg VSS)	Dry weight (mg/mL)	Colonies (CFU/mL)	α (CFU/mg)
7	0.122	366.7	3.23	2.63E+08	8.14E+07
8	0.100	219.7	1.68	4.87E+06	2.89E+06

The results presented in Table 5.19, regarding the biofilm quantification are in accordance to the other results presented, since as can be seen, the biofilm formed with the thinner membrane show a higher dry weight and a higher amount of microorganism per biofilm dry weight. This leads to a thicker, more dense and active biofilm increasing the substrate degradation, the electrons transfer rate and consequently the fuel cell performance.

Based on the results presented, it is possible to conclude that the use of thinner membranes improves the cell performance and allows to achieve higher power density and substrate degradation rates. Also, thinner membranes have lower costs, a very important factor to have in consideration for scale-up MFCs.

5.4 Concluding remarks

The performance of a microbial fuel cell operating with *Lactobacillus pentosus* has been studied to systematically evaluate the effects of configurational parameters, such as membrane thickness and area, anode electrode size, reactor design, operating mode and yeast extract concentration on the MFC power output, COD removal rate and biofilmformationon the anode side.

Based on the results presented in this chapter, it can be concluded that the overall performance of the MFC significantly increases with the operation in batch mode. However, the continuous mode is more adequate for large scale operations and achieved a maximum power density of 5.36 mW/m^2.

Considering the power density achieved it is possible to state that the dual chamber MFC presents the best performance. However, considering the wastewater treatment aspect, the single chamber configuration allows better substrate degradation, so lower COD removal rates. The use of a single chamber is also more favorable to practical applications since the reactor configuration is simpler.

Regarding the membrane area, the higher power density was achieved with the lower membrane area (5.44 mW/m^2). For the wastewater treatment the best results were obtained by the cell with the higher membrane area, achieving a better substrate degradation.The use of a lower membrane presents the advantage of being less expensive.

Higher anode electrodes have higher power densities, but lower COD removal rates were achieved. Concluding, it was possible to verify that a higher anode electrode leads to a higher energy production but worse wastewater treatment.

Concerning the yeast extract amount it was possible to conclude that higher concentrations lead to higher power densities and better substrate degradation witha maximum COD removal rate of 68%.

The use of thinner membranes allows to achieve better power densities, substrate degradation rates and ticker biofilms. Also, it have lower costs than the thicker ones.

6 Conclusions

Many studies have been carried out in order to improve and develop the power generation by Microbial Fuel Cells. A lot of parameters affect the electrons transport from the anode to the cathode and the substrate oxidation, affecting the power outputs and the wastewater treatment. Many efforts have been made to understand and explain their effects on MFC performance and due to that the studies regarding the MFC are increasing. By the optimization of the operating and configuration conditions it is possible to find optimized conditions and make progress in the developments of this technology. In order to study some of these parameters, different tests were performed in a lab scale Microbial Fuel Cell. Studies were performed in order to compare the operation mode (continuous mode versus batch mode), and for each mode different configurations were tested. For the continuous mode the following effects were tested: cell configuration, membrane area and anode electrode area. For the batch mode were studied the effect of the quantity of the yeast extract and the effect of the membrane thickness. The Microbial Fuel Cell performance was evaluated base on the polarization curves, the power densities, the COD removal values, the biofilm characterization and other parameters that were monitored throughout each experiment.

To study the **effect of the operation** mode tests in continuous and in batch mode were performed. It was possible to reach a higher power density for the continuous mode with a value of 5.36 mW/m^2, and a power density of 5.00 mW/m^2 for the batch mode. The COD removal rate was similar to both assays, achieving a maximum value of 66%. However, the concentration of sugar on the biofilm was lower for the batch mode indicating a better substrate degradation in this case. Due to the higher hydraulic retention time, the biofilm formed in the batch mode presented greater thickness and a higher amount of bacteria, representing a more active biofilm and a higher electrons transfer rate.

The **effect of the cell configuration** was studied by performing tests ina dual and single chamber MFCs. The higher power density was achieved for dual chamber and was 10.7 mW/m^2. The COD removal rate was above the 58% for the single chamber and for the dual chamber was achieved a maximum of 73%, in the last week, but all the other values are lower than 50%. Concluding, the dual chamber presents the best performance but the single chamber allows better substrate degradation.

Two membranes with different areas were used to test the **effect of the membrane area**, on the MFC performance. For low current densities the higher membrane presented better results, but for higher current densities the membrane with lower area was better. The biofilm formed with the higher membrane area presented a higher thickness and a higher activity. This can be a disadvantage for high current densities due to mass transport limitations on the electrode surface. The lower membrane area achieved a power density of 5.44 mW/m^2 and the other 4.73 mW/m^2. The MFC with a higher membrane area had better substrate degradation and greater COD removal rates.

For the **effect of the anode electrode area**it was possible to notice that the higher anode electrode had a better performance. The power densities were greater in every polarization curves performed. However, the higher COD removal rates were achieved with the lower electrode, with a maximum of 74%. In sum, a lower anode leads to a better wastewater treatment but a worst power output.

To study the **amount of yeast extract** two different concentrations were used. Despite the major voltage value reached for the test with the normal quantity of yeast extract the power outputs for medium and higher current densities were higher for the MFC tested with a higher concentration of yeast extract. This condition also leads to better substrate degradation with a maximum COD removal of 68%.

The **effect of the membrane thickness** was evaluated by using two different membrane thicknesses, the Nafion 117and Nafion 212. Thinner membrane presented a better performance, achieving power densities of 5.00 mW/m^2, COD removal rates of 66% and thicker, denser and more active biofilms.

The results presented, in this work, showed that changes in the MFC configuration and operating modes constitute effective ways to increase the MFC power output, substrate degradation rates and biofilm characteristics. It was also, found that, in some cases one parameter presents the best overall performance, but others reach higher power output and others achieve a more efficient wastewater treatment.

7 Evaluation of the developed work

7.1 Goals Achieved

As mentioned, the main goal of this work was to study the effect of different configurations of a Microbial Fuel Cell on its overall performance (energy production, wastewater treatment and biofilm formation). In order to achieve that, different test were carried on the MFC. Experiments were performed to study the best operation mode, continuo versus batch and for each operating mode various configurations were tested.

All the experimental work was accomplish and the objectives for each testwere achieved. The results presented show that this thesis objectives were fulfil.

7.2 Limitations and Future work

Besides the studies performed and the advances achieved in the last years, a lot of work needs to be done in order to improve this technology towards its introduction in the market. During the realization of the present work it was possible to identify some limitations of working with microorganisms in MFC. Some of these limitations are:

- Control of the parameters being monitored and affect the MFC performance, like pH and temperature;
- Prevention of contamination by other microorganisms;
- Control of the anaerobic conditions in the anode chamber;
- Possibility of pipes obstruction;

Many things can be done in order to support the results already obtained and to increase the knowledge and development in MFCs. Some aspects to further develop in future work are:

- Performexperiments with more controlled conditions, as operation in anaerobic chamber, addition of buffers to control the pH variations and temperature control;
- Study the effect of the distance between electrodes;
- More detailed study of the biofilm growth, perform biofilm quantification in each polarization curvemeasurement;
- Deep study of the bacteria growth during the operation time of the MFC, develop tri-dimensional graphics describing the bacteria growth and relate it with the parameters that influence their evolution;

- Evaluate the MFC performance with a bacteria consortium and the effluent used in this work;
- Study the effect of the cathode electrode area;
- Study the effect of the anode/cathode electrode materials;

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9 Appendixes

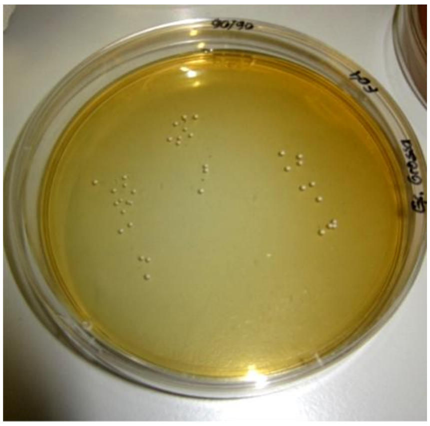
9.1 Appendix A- Microbial Plating

This technique aims to quantify *Lactobacillus pentosus* colonies presented in the samples. The plating was made in medium MRS (specific medium) and different dilutions were made during the experiments to quantify the number of bacteria or to see if there was any contamination. The procedure is described below:

Method:

- Sample collection;
- Light the flame and perform all the dilutions and the plating near the flame;
- Pipette 1 mL of the sample and transfer to the first tube and mix it in the vortex;
- Pipette 1 mL of the first tube to the second one and repeat the procedure until the number of dilutions needed;
- After the dilutions pipette 30 µL 3 times for 3 different sections in the same plate of MRS, spread the sample in each section to obtain 3 replicates;
- Incubate the sample during two days.

Example of a plating made during the experiments:



9.1 – Example of a plating of the sample.

9.2 Appendix B - Chemical Oxygen Demand (COD)

This test gives us indirectly the concentration of organic matter evaluated based on the quantity of oxygen that is used to oxidize the organic matter presented in the sample by the application of this test: the chemical oxygen demand test (Logan, 2008).

This test could be done using different methods. In our case was done with the application of the potassium dichromate reflux method. Since this test was done a lot of times during the experiments it presented some advantages in the handling and application. The procedure performed is explained next:

Method:

- Pipette 3.5 mL of sulfuric acid reagent (acting as oxidant) for a glass tube appropriated to this analysis;
- Pipette to the same tube 1.5 mL of potassium dichromate digestion solution;
- Dilution the sample [1];
- Added 2.5 mL of the sample to the tubes prepared;
- Heat the sample for 2 hours at 150 ºC in the digestor;
- After cooling read the values in the photometer.

Three replicates were done to minimize the errors associated to the equipment used and to achieve a medium value.

[1] The samples were centrifuged at 4000 rpm during 15 minutes, and the dilution factor was adjusted to the sample.

9.2 – Samples prepared for digestion – COD.

9.3 Appendix C- Sugars Quantification - Dubois Method

The following method was performed to control the variations on the sugar concentrations.

Method:

- Put 250 µL of the sample in the assay glass tube (do some dilution if necessary);
- Add 0.5 mL of phenol and more 2.5 mL of sulfuric acid;
- Vortex the sample inside the hotte;
- Wait 5 minutes to read;
- Use a glass cuvette to read the absorbance at 490 nm;
- Do a read of the white sample (with distillated water) and after that do all the others reads;
- Use the calibration curve to obtain the sugar concentration values;

The calibration curve used is:

$$y = 0.0037x + 0.00512$$

Where x represents the sugar concentration values (in mg/L) and y the absorbance values.

9.4 Appendix D – Biofilm extraction and quantification

Extraction method:

- Add the extraction protein solution (EPS) to dilute the sample of biofilm in the brush until 20 mL of the falcon tube;
- Desegregate the biofilm of the brush filaments using the vortex;
- Put the sample in a beakerwith 2 g of resin Dowex (Naform, strongly acidic, 20-50 mesh, Sigma-Aldrich, Portugal) ;
- The extraction occurs at 400 rpm and 4ºC during 4 hours;
- After the resin separation, centrifuge the sample at 4000 rpm during 15 minutes;
- Separation of the biomass (pellet) of the surrounding substance (Matrix);
- Fill the tube of the pellet with EPS until the level with both samples (Pellet + Matrix)
- Quantification of the pellet and the matrix;

9.3 – Preparation of the biofilm desegregation.

Biofilm Mass quantification

Biomass quantification: the dry mass of the biofilms is assessed by the determination of the total volatile solids (TVS) of the homogenized biofilm suspensions, according to Standard Methods:

- Dry the crucibles in the oven at 550 ± 5°C for 2h;
- Cool in a desiccator (30'- 45') and weigh (C)
- Transfer 10 mL of the homogenized suspension to the crucible (identified)
- Place in an oven at 110 ± 2°C for 24h
- Cool in a desiccator (30'- 45') and weigh (A)
- Place in an furnace at 550 ± 5°C for 2h
- Cool in a desiccator (1h) and weigh (B)
- Dry weight = A-B

9.5 Appendix E - Total Protein Kit, Micro Lowry, Peterson's modification for protein quantification

The following method was used to characterize the biofilm.

Method:

- Put 250 µL of the sample in each eppendorf (if necessary do some dilutions);
- Add 250 µL of Lowry Reagent Solution;
- Let it stand during 20 minutes at room temperature;
- Add 125 µL of Folin & Ciocalteu's Phenol ReagentWorking Solution;
- Wait 30 minutes at room temperature;
- Read the absorbance in a plastic cuvette at 595 nm;
- Do a read of the white sample (with distillated water) and after that do all the others reads;
- Use the calibration curve to obtain the protein concentration values;

The calibration curve used is:

$$y = 0.0048x + 0.2535$$

Where *x* is the protein concentration values (in mg/L) and *y* the absorbance values.

9.6 Appendix F- Operation of the MFC in continuous mode: results and polarization curves

Table 9.1 – MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
1	Dual	Continuous	BP 1"	25 cm^2	Nafion 212	5

Table 9.2 - Experimental values of voltage vs current density – 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.547	0.00
0.01	4	0.516	2.06
0.02	8	0.474	3.79
0.03	12	0.436	5.23
0.04	16	0.393	6.29
0.05	20	0.331	6.62
0.06	24	0.261	6.26
0.07	28	0.231	6.47
0.08	32	0.102	3.26
0.09	36	0.015	0.54
0.1	40	0.000	0.00
0.05	20	0.331	6.62

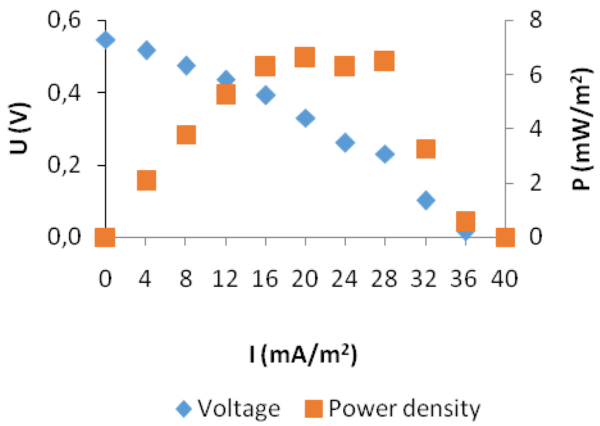


Figure 9.4 - 1st polarization curve.

Table 9.3 - Experimental values of voltage vs current density - 2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.649	0.00
0.01	4	0.617	2.47
0.02	8	0.555	4.44
0.03	12	0.505	6.06
0.04	16	0.444	7.10
0.05	20	0.399	7.98
0.06	24	0.368	8.83
0.07	28	0.360	10.08
0.08	32	0.318	10.18
0.09	36	0.288	10.37
0.1	40	0.258	10.32
0.11	44	0.220	9.68
0.12	48	0.185	8.88
0.13	52	0.141	7.33
0.14	56	0.097	5.43
0.15	60	0.050	3.00
0.16	64	0.017	1.09
0.09	36	0.288	10.37

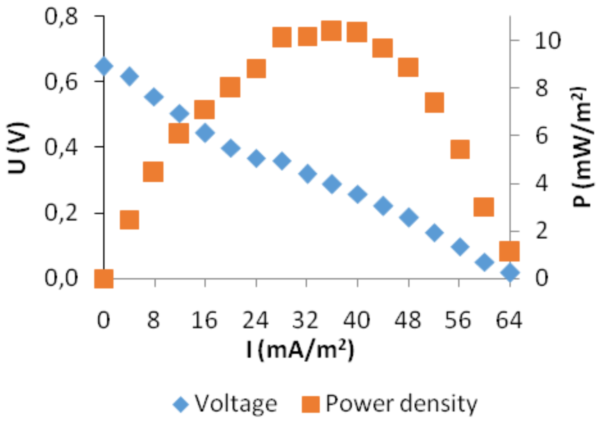


Figure 9.5 - 2nd polarization curve.

Table 9.4 - Experimental values of voltage vs current density - 3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.822	0.00
0.01	4	0.770	3.08
0.02	8	0.763	6.10
0.03	12	0.611	7.33
0.04	16	0.518	8.29
0.05	20	0.429	8.58
0.06	24	0.343	8.23
0.07	28	0.265	7.42
0.08	32	0.188	6.02
0.09	36	0.111	4.00
0.1	40	0.044	1.76
0.11	44	0.015	0.66
0.05	20	0.429	8.58

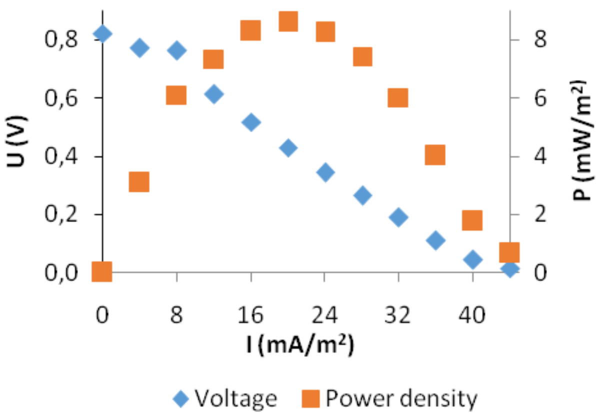


Figure 9.6 - 3rd polarization curve.

Table 9.5 - Experimental values of voltage vs current density - 4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.730	0.00
0.01	4	0.677	2.71
0.02	8	0.600	4.80
0.03	12	0.517	6.20
0.04	16	0.424	6.78
0.05	20	0.333	6.66
0.06	24	0.250	6.00
0.07	28	0.169	4.73
0.08	32	0.057	1.82
0.09	36	0.008	0.29
0.04	16	0.424	6.78

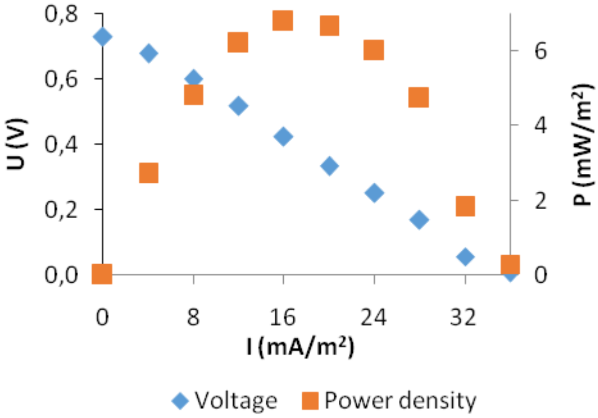


Figure 9.7 - 4th polarization curve.

Table 9.6- MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
2	Dual	Continuous	BP 3/4"	25 cm^2	Nafion 212	5

Table 9.7- Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.246	0.00
0.01	4	0.015	0.06
0.01	4	0.015	0.06

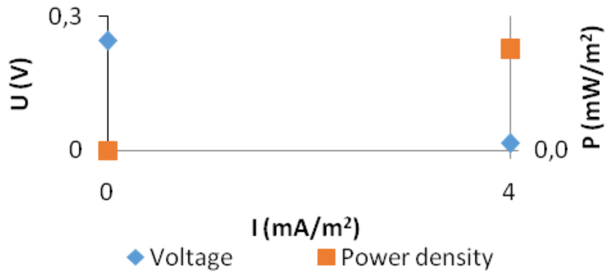


Figure 9.8- 1st polarization curve.

Table 9.8- Experimental values of voltage vs current density - 2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.616	0.00
0.01	4	0.499	2.00
0.02	8	0.431	3.45
0.03	12	0.358	4.30
0.04	16	0.32	5.12
0.05	20	0.272	5.44
0.06	24	0.191	4.58
0.07	28	0.13	3.64
0.08	32	0.07	2.24
0.09	36	0.015	0.54
0.05	20	0.272	5.44

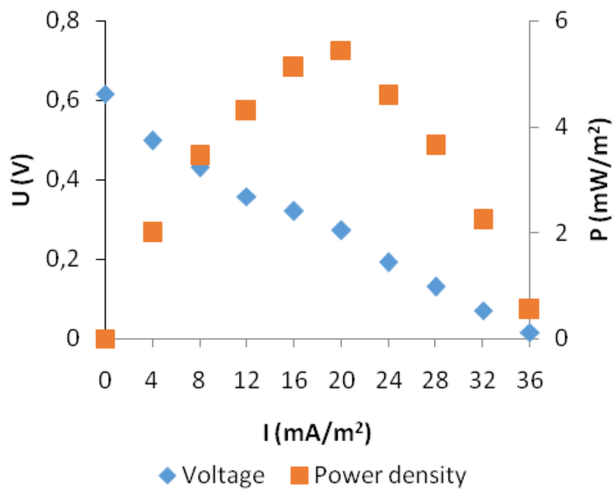


Figure 9.9 - 2ndpolarization curve.

Table 9.9 - Experimental values of voltage vs current density - 3rd polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.71	0.00
0.01	4	0.472	1.89
0.02	8	0.250	2.00
0.03	12	0.009	0.11
0.02	8	0.250	2.00

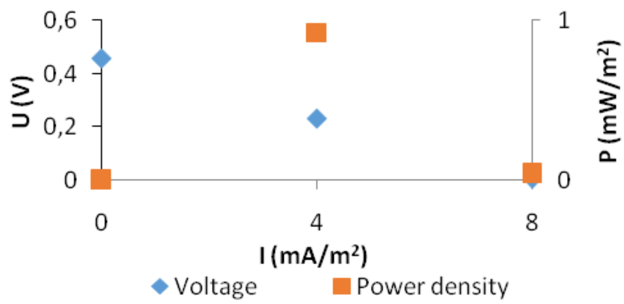


Figure 9.10 - 3rd polarization curve.

Table 9.10 - Experimental values of voltage vs current density - 4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.71	0.00
0.01	4	0.472	1.89
0.02	8	0.250	2.00
0.03	12	0.009	0.11
0.02	8	0.250	2.00

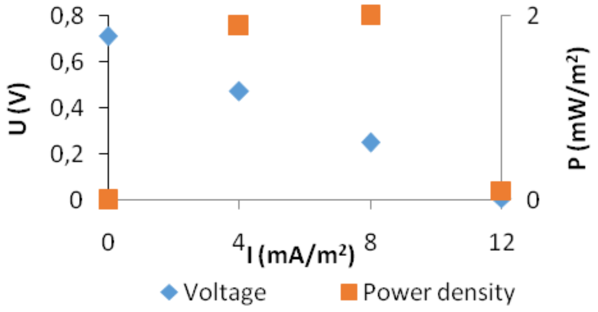


Figure 9.11 - 4th polarization curve.

Table 9.11-MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
3	Dual	Continuous	BP 1"	42,25 cm^2	Nafion 212	5

Table 9.12- Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.715	0.00
0.01	2.37	0.616	1.46
0.02	4.73	0.482	2.28
0.03	7.10	0.334	2.37
0.04	9.47	0.174	1.65
0.05	11.83	0.015	0.18
0.03	7.10	0.33	2.37

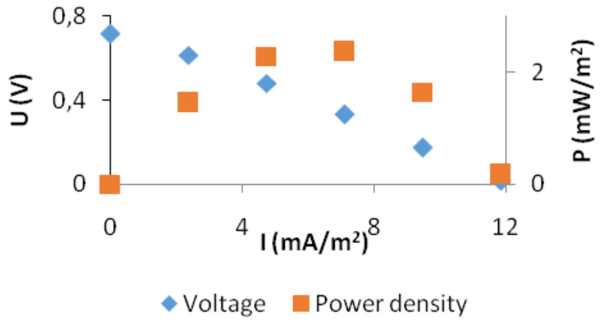


Figure 9.12 - 1st polarization curve.

Table 9.13- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.773	0.00
0.01	2.37	0.708	1.68
0.02	4.73	0.579	2.74
0.03	7.10	0.443	3.15
0.04	9.47	0.308	2.92
0.05	11.83	0.17	2.01
0.06	14.20	0.038	0.54
0.07	16.57	0.013	0.22
0.03	7.10	0.44	3.15

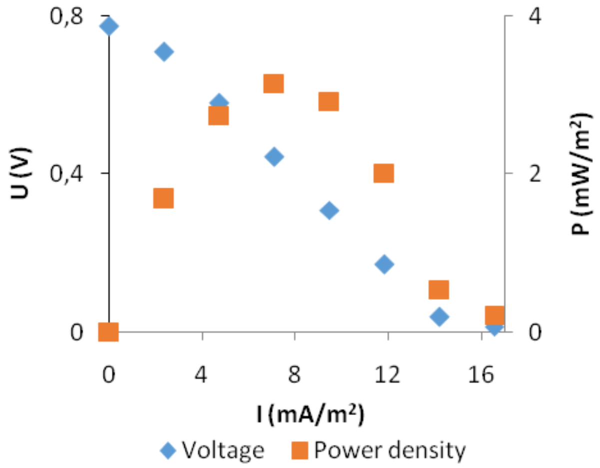


Figure 9.13 - 2nd polarization curve.

Table 9.14 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.74	0.00
0.01	2.37	0.658	1.56
0.02	4.73	0.516	2.44
0.03	7.10	0.355	2.52
0.04	9.47	0.197	1.87
0.05	11.83	0.034	0.40
0.06	14.20	0.008	0.11
0.03	7.10	0.36	2.52

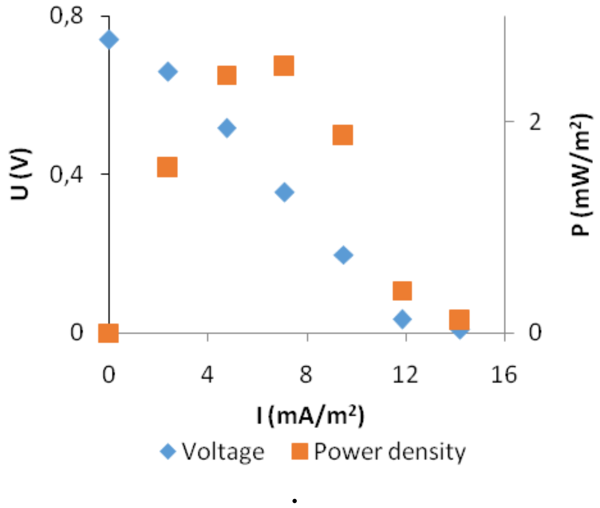


Figure 9.14 - 3rd polarization curve.

Table 9.15 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.736	0.00
0.01	2.37	0.641	1.52
0.02	4.73	0.478	2.26
0.03	7.10	0.323	2.29
0.04	9.47	0.153	1.45
0.05	11.83	0.011	0.13
0.03	7.10	0.32	2.29

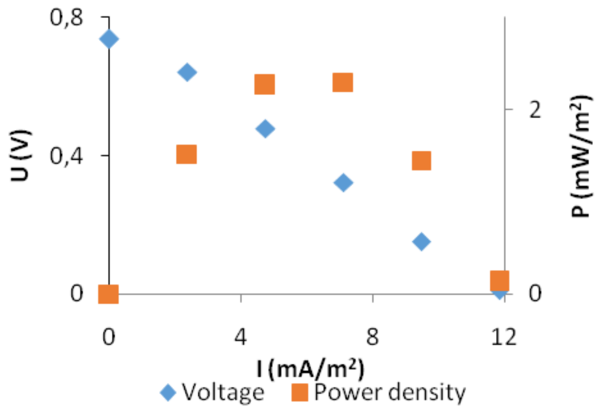


Figure 9.15- 4th polarization curve.

Table 9.16–MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
4	Dual	Continuous	BP 3/4"	42,25 cm^2	Nafion 212	5

Table 9.17- Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.723	0.00
0.01	2.37	0.460	1.09
0.02	4.73	0.204	0.97
0.03	7.10	0.010	0.07
0.01	2.37	0.46	1.09

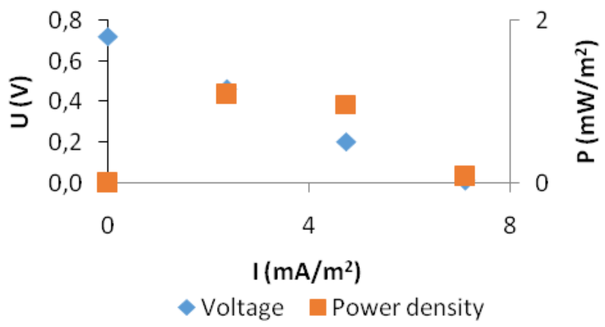


Figure 9.16 - 1st polarization curve.

Table 9.18- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.719	0.00
0.01	2.37	0.463	1.10
0.02	4.73	0.205	0.97
0.03	7.10	0.010	0.07
0.01	2.37	0.463	1.10

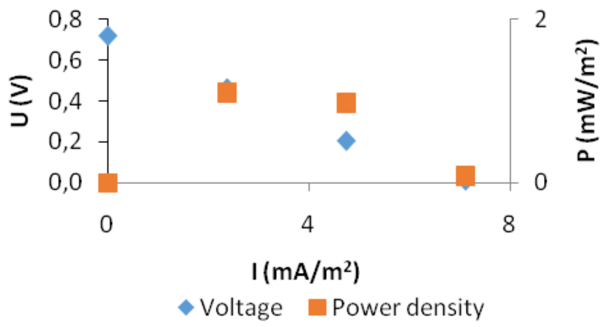


Figure 9.17 - 2nd polarization curve.

Table 9.19 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.755	0.00
0.01	2.37	0.594	1.41
0.02	4.73	0.388	1.84
0.03	7.10	0.154	1.09
0.04	9.47	0.010	0.09
0.02	4.73	0.39	1.84

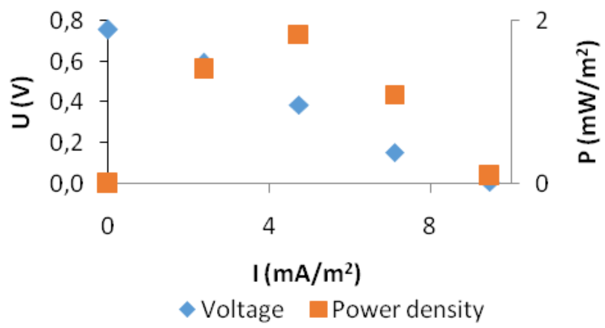


Figure 9.18 - 3rd polarization curve.

Table 9.20 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0.00	0.293	0.00
0.01	2.37	0.078	0.18
0.02	4.73	0.005	0.02
0.01	2.37	0.078	0.18

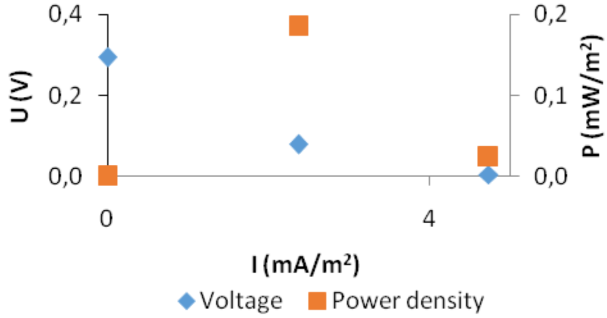


Figure 9.19- 4th polarization curve.

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Table 9.21-MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
5	Single	Continuous	BP 1"	25 cm^2	Nafion 212	5

Table 9.22 - Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.890	0.00
0.01	4	0.738	2.95
0.02	8	0.559	4.47
0.03	12	0.447	5.36
0.04	16	0.274	4.38
0.05	20	0.092	1.84
0.06	24	0.008	0.19
0.03	12	0.447	5.36

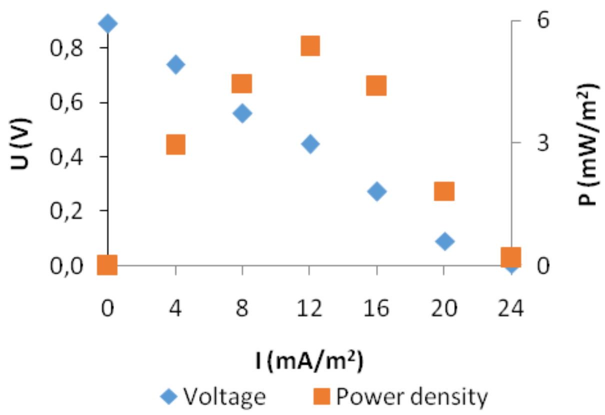

Figure 9.20- 1st polarization curve.

Table 9.23- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.811	0.00
0.01	4	0.622	2.49
0.02	8	0.404	3.23
0.03	12	0.2	2.40
0.04	16	0.019	0.30
0.05	20	0.003	0.06
0.02	8	0.40	3.23

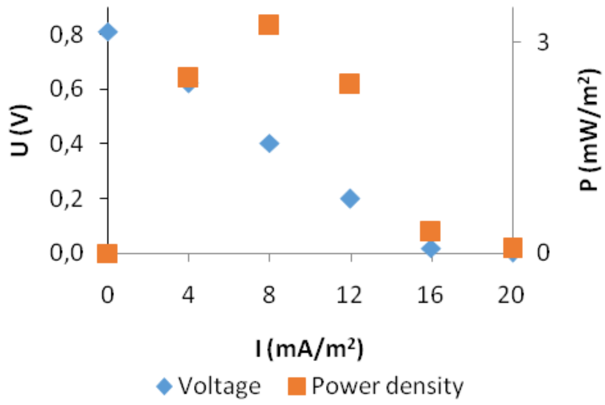

Figure 9.21 - 2nd polarization curve.

Table 9.24 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.749	0.00
0.01	4	0.568	2.27
0.02	8	0.359	2.87
0.03	12	0.152	1.82
0.04	16	0.010	0.16
0.02	8	0.359	2.87

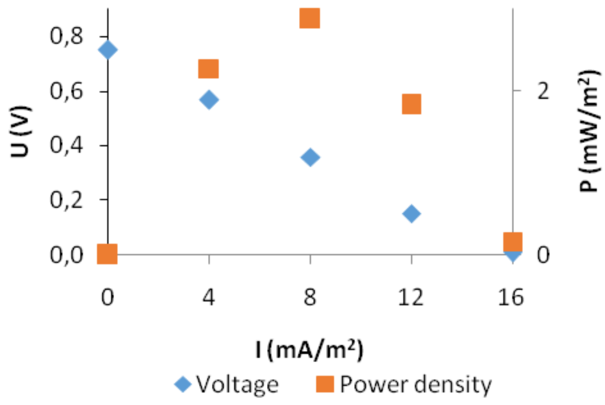

Figure 9.22 - 3rd polarization curve.

Table 9.25 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.982	0.00
0.01	4	0.613	2.45
0.02	8	0.337	2.70
0.03	12	0.192	2.30
0.04	16	0.075	1.20
0.05	20	0.007	0.14
0.02	8	0.34	2.70

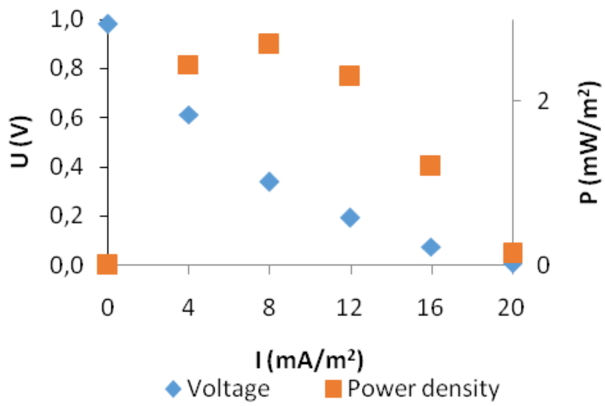

Figure 9.23- 4th polarization curve.

Table 9.26-MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
6	Single	Continuous	BP 3/4"	25 cm^2	Nafion 212	5

Table 9.27 - Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.782	0.00
0.01	4	0.464	1.86
0.02	8	0.123	0.98
0.03	12	0.007	0.08
0.01	4	0.464	1.86

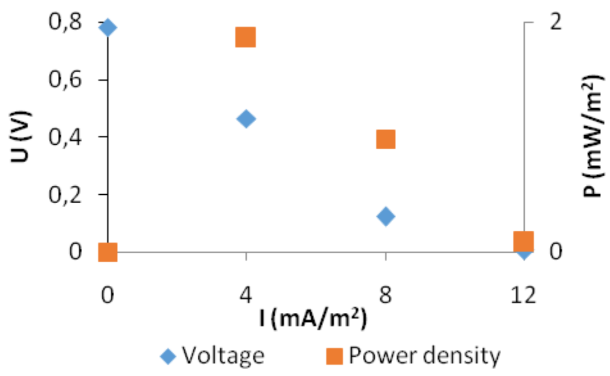

Figure 9.24- 1st polarization curve.

Table 9.28- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.693	0.00
0.01	4	0.495	1.98
0.02	8	0.259	2.07
0.03	12	0.032	0.38
0.04	16	0.007	0.11
0.02	8	0.259	2.07

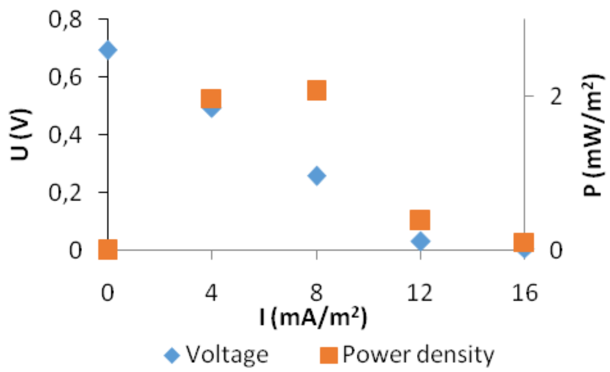

Figure 9.25 - 2nd polarization curve.

Table 9.29 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.635	0.00
0.01	4	0.463	1.85
0.02	8	0.225	1.80
0.03	12	0.009	0.11
0.01	4	0.463	1.85

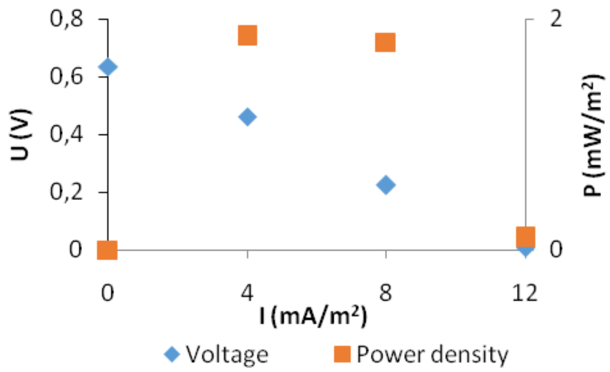

Figure 9.26 - 3rd polarization curve.

Table 9.30 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.881	0.00
0.01	4	0.702	2.81
0.02	8	0.482	3.86
0.03	12	0.285	3.42
0.04	16	0.086	1.38
0.05	20	0.007	0.14
0.02	8	0.482	3.86

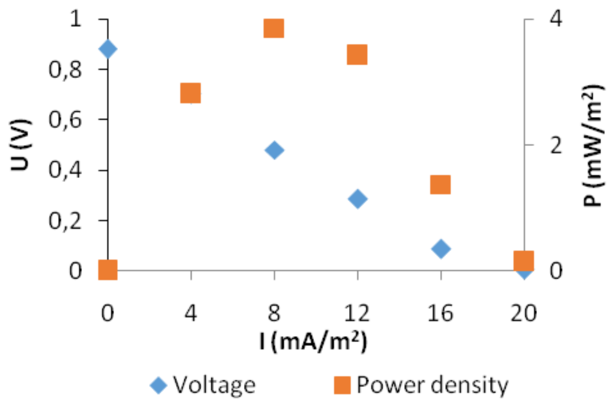

Figure 9.27- 4th polarization curve.

9.7 Appendix G- Operation of the MFC in batch: results and polarization curves

Table 9.31-MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
7	Single	Batch	BP 1"	25 cm^2	Nafion 212	5

Table 9.32 - Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.876	0.00
0.01	4	0.740	2.96
0.02	8	0.530	4.24
0.03	12	0.372	4.46
0.04	16	0.150	2.40
0.05	20	0.010	0.20
0.03	12	0.372	4.46

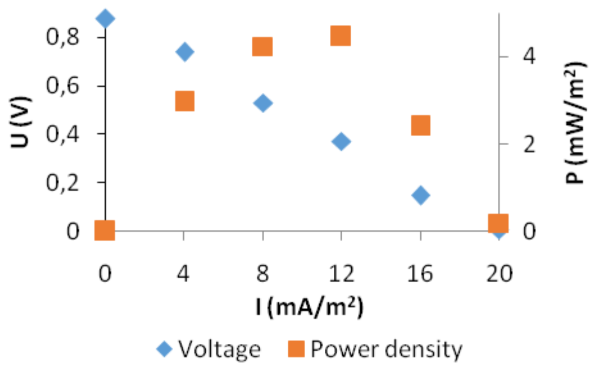

Figure 9.28 - 1st polarization curve.

Table 9.33- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.921	0.00
0.01	4	0.777	3.11
0.02	8	0.586	4.69
0.03	12	0.417	5.00
0.04	16	0.197	3.15
0.05	20	0.007	0.14
0.03	12	0.417	5.00

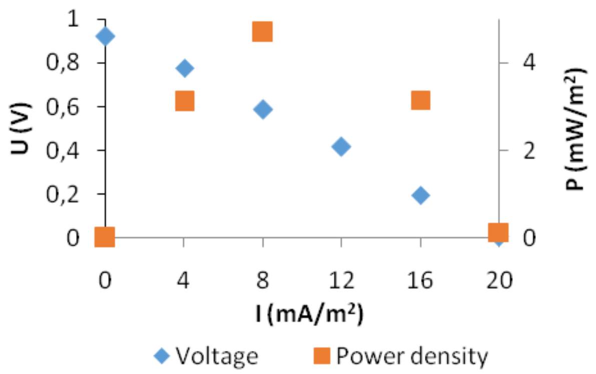

Figure 9.29 - 2nd polarization curve.

Table 9.34 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.913	0.00
0.01	4	0.754	3.02
0.02	8	0.551	4.41
0.03	12	0.347	4.16
0.04	16	0.12	1.92
0.05	20	0.005	0.10
0.02	8	0.551	4.41

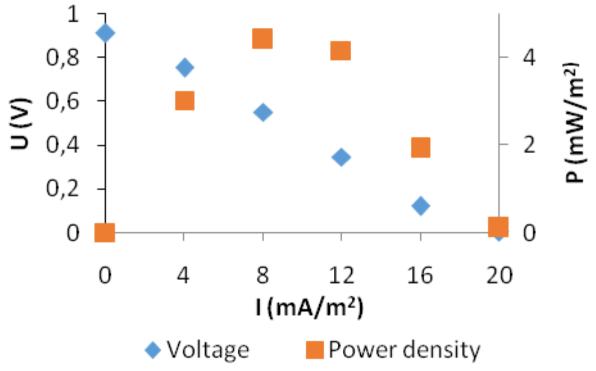

Figure 9.30 - 3rd polarization curve.

Table 9.35 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.650	0.00
0.01	4	0.552	2.21
0.02	8	0.411	3.29
0.03	12	0.268	3.22
0.04	16	0.109	1.74
0.05	20	0.005	0.10
0.02	8	0.411	3.29

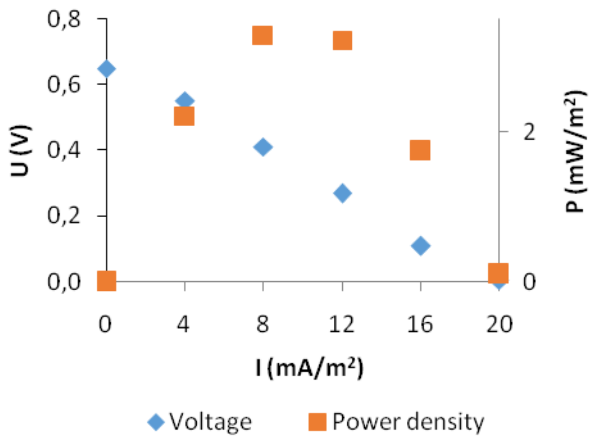

Figure 9.31- 4th polarization curve.

Table 9.36 - Experimental values of voltage vs current density -5th polarization.

5th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.611	0.00
0.01	4	0.553	2.21
0.02	8	0.399	3.19
0.03	12	0.245	2.94
0.04	16	0.075	1.20
0.05	20	0.01	0.20
0.02	8	0.399	3.19

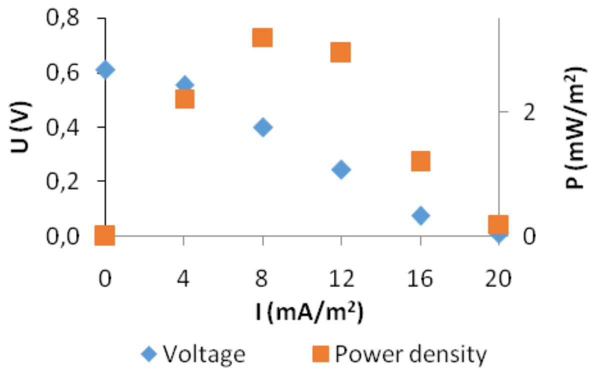

Figure 9.32- 5th polarization curve.

Table 9.37 - Experimental values of voltage vs current density -6th polarization.

6th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.709	0.00
0.01	4	0.591	2.36
0.02	8	0.428	3.42
0.03	12	0.265	3.18
0.04	16	0.086	1.38
0.05	20	0.005	0.10
0.02	8	0.428	3.42

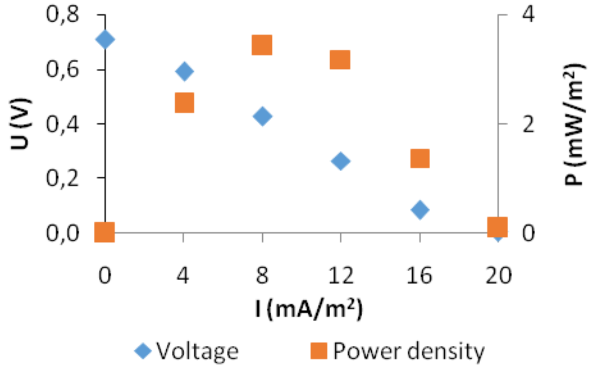

Figure 9.33- 6th polarization curve.

Table 9.38 - Experimental values of voltage vs current density -7th polarization.

7th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.664	0.00
0.01	4	0.561	2.24
0.02	8	0.413	3.30
0.03	12	0.254	3.05
0.04	16	0.072	1.15
0.05	20	0.01	0.20
0.02	8	0.413	3.30

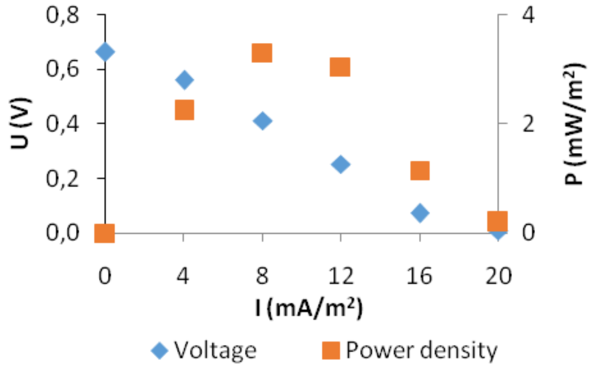

Figure 9.34- 7th polarization curve.

Table 9.39–MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
8	Single	Batch	BP 1"	25 cm^2	Nafion 117	5

Table 9.40 - Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.661	0.00
0.01	4	0.500	2.00
0.02	8	0.338	2.70
0.03	12	0.191	2.29
0.04	16	0.030	0.48
0.05	20	0.010	0.20
0.02	8	0.338	2.70

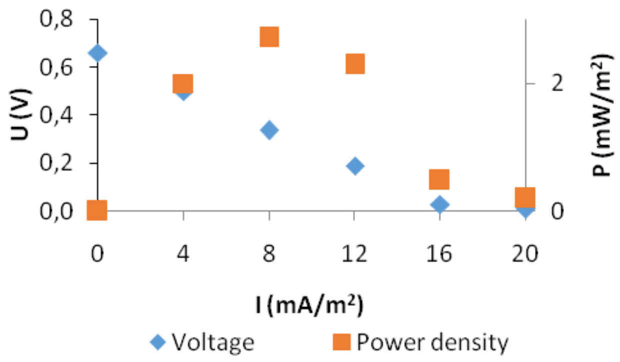

Figure 9.35 - 1st polarization curve.

Table 9.41- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.692	0.00
0.01	4	0.49	1.96
0.02	8	0.315	2.52
0.03	12	0.133	1.60
0.04	16	0.003	0.05
0.02	8	0.315	2.52

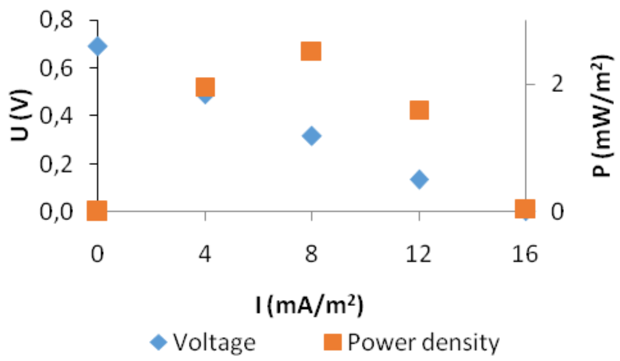

Figure 9.36 - 2nd polarization curve.

Table 9.42 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.698	0.00
0.01	4	0.501	2.00
0.02	8	0.314	2.51
0.03	12	0.142	1.70
0.04	16	0.010	0.16
0.02	8	0.314	2.51

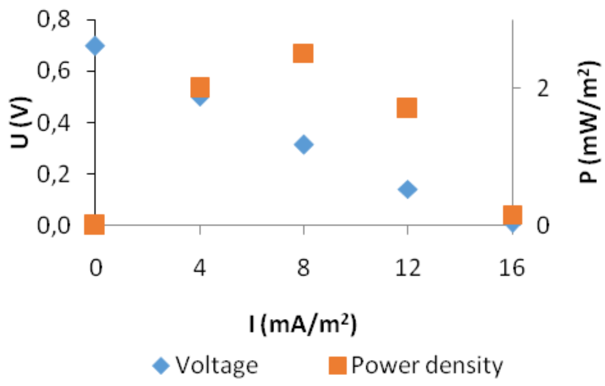

Figure 9.37 - 3rd polarization curve.

Table 9.43 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.715	0.00
0.01	4	0.509	2.04
0.02	8	0.314	2.51
0.03	12	0.120	1.44
0.04	16	0.010	0.16
0.02	8	0.314	2.51

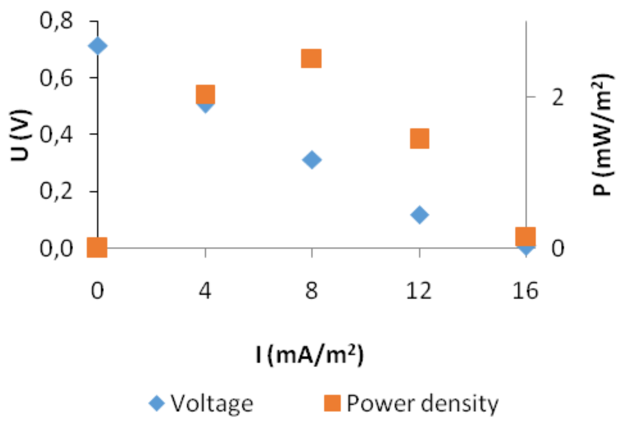

Figure 9.38- 4th polarization curve.

Table 9.44 - Experimental values of voltage vs current density -5th polarization.

5th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.679	0.00
0.01	4	0.486	1.94
0.02	8	0.297	2.38
0.03	12	0.12	1.44
0.04	16	0.005	0.08
0.02	8	0.297	2.38

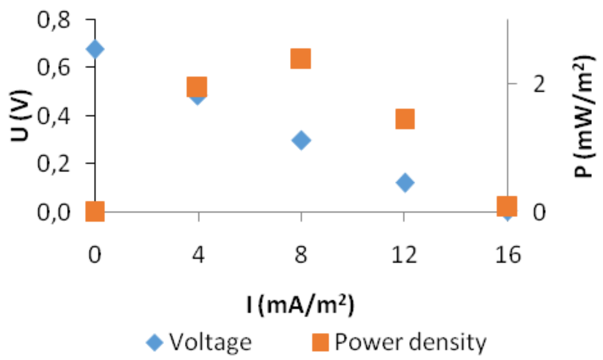

Figure 9.39- 5th polarization curve.

Table 9.45 - Experimental values of voltage vs current density -6th polarization.

6th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.644	0.00
0.01	4	0.467	1.87
0.02	8	0.289	2.31
0.03	12	0.123	1.48
0.04	16	0.005	0.08
0.02	8	0.289	2.31

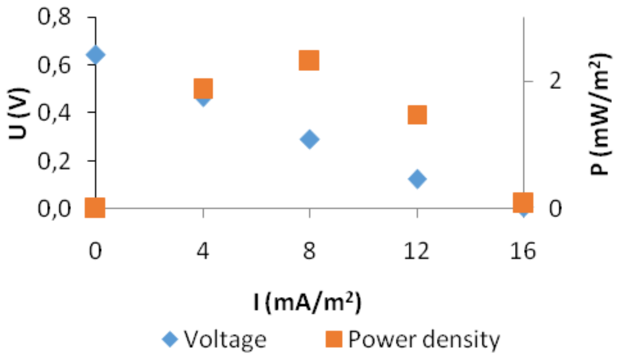

Figure 9.40- 6th polarization curve.

Table 9.46 - Experimental values of voltage vs current density -7th polarization.

7th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.619	0.00
0.01	4	0.462	1.85
0.02	8	0.295	2.36
0.03	12	0.139	1.67
0.04	16	0.005	0.08
0.02	8	0.295	2.36

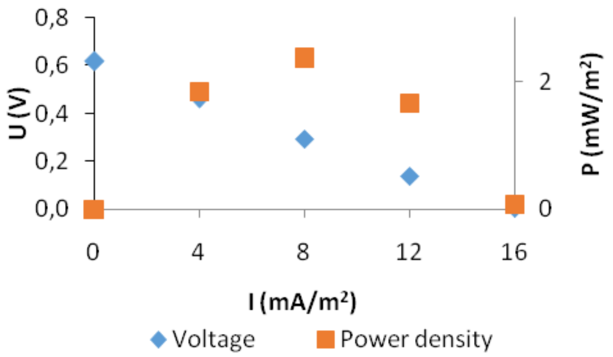

Figure 9.41- 7th polarization curve.

Table 9.47-MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
9	Single	Batch	BP 1"	25 cm^2	Nafion 212	50

Table 9.48 - Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.798	0.00
0.01	4	0.672	2.69
0.02	8	0.505	4.04
0.03	12	0.338	4.06
0.04	16	0.155	2.48
0.05	20	0.008	0.16
0.03	12	0.338	4.06

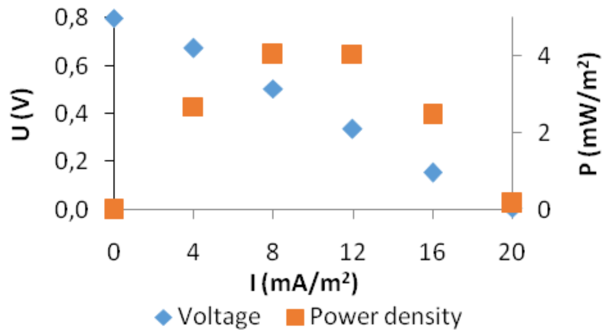

Figure 9.42 - 1st polarization curve.

Table 9.49- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.804	0.00
0.01	4	0.683	2.73
0.02	8	0.523	4.18
0.03	12	0.355	4.26
0.04	16	0.17	2.72
0.05	20	0.008	0.16
0.03	12	0.355	4.26

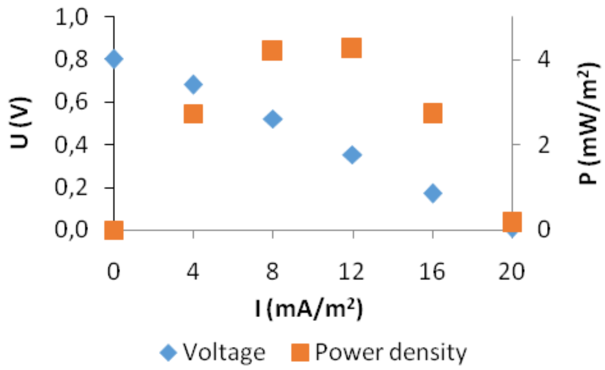

Figure 9.43 - 2nd polarization curve.

Table 9.50 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m²)	U (V)	P (mW/m²)
0	0	0.811	0.00
0.01	4	0.701	2.80
0.02	8	0.551	4.41
0.03	12	0.392	4.70
0.04	16	0.216	3.46
0.05	20	0.035	0.70
0.06	24	0.008	0.19
0.03	12	0.392	4.70

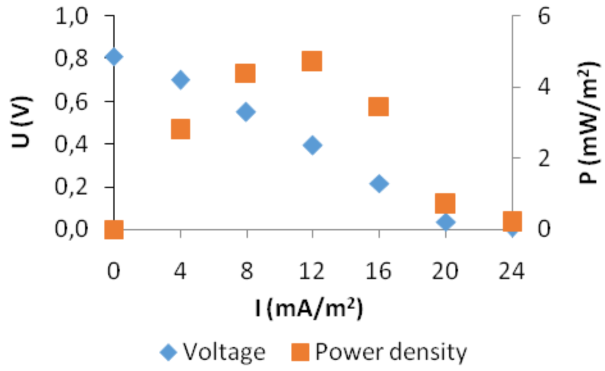

Figure 9.44 - 3rd polarization curve.

Table 9.51 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m²)	U (V)	P (mW/m²)
0	0	0.834	0.00
0.01	4	0.73	2.92
0.02	8	0.575	4.60
0.03	12	0.419	5.03
0.04	16	0.240	3.84
0.05	20	0.058	1.16
0.06	24	0.008	0.19
0.03	12	0.419	5.03

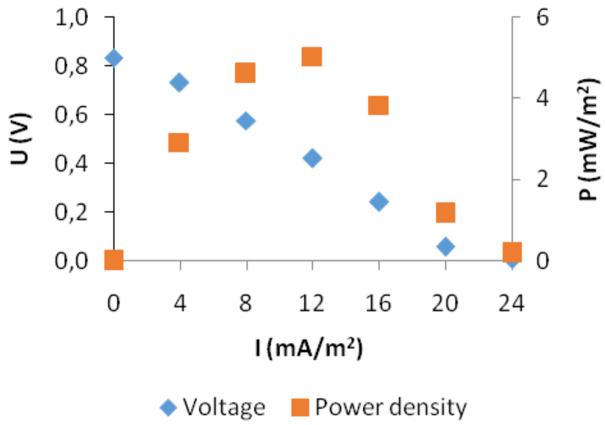

Figure 9.45- 4th polarization curve.

Table 9.52 - Experimental values of voltage vs current density -5th polarization.

5th Polarization			
I (mA)	I (mA/m²)	U (V)	P (mW/m²)
0	0	0.862	0.00
0.01	4	0.761	3.04
0.02	8	0.615	4.92
0.03	12	0.461	5.53
0.04	16	0.286	4.58
0.05	20	0.112	2.24
0.06	24	0.008	0.19
0.03	12	0.461	5.53

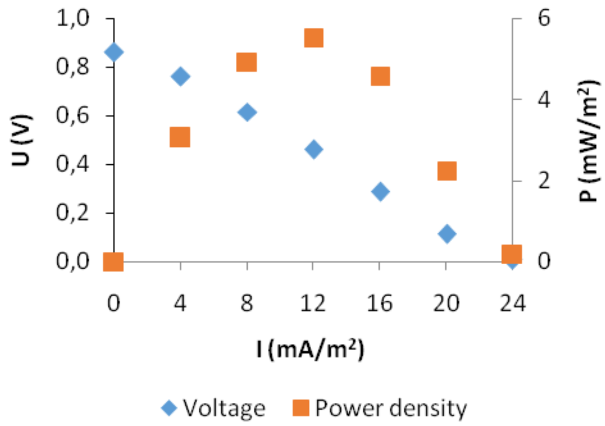

Figure 9.46- 5th polarization curve.

Table 9.53 - Experimental values of voltage vs current density -6th polarization.

6th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.818	0.00
0.01	4	0.724	2.90
0.02	8	0.582	4.66
0.03	12	0.443	5.32
0.04	16	0.285	4.56
0.05	20	0.117	2.34
0.06	24	0.008	0.19
0.03	12	0.443	5.32

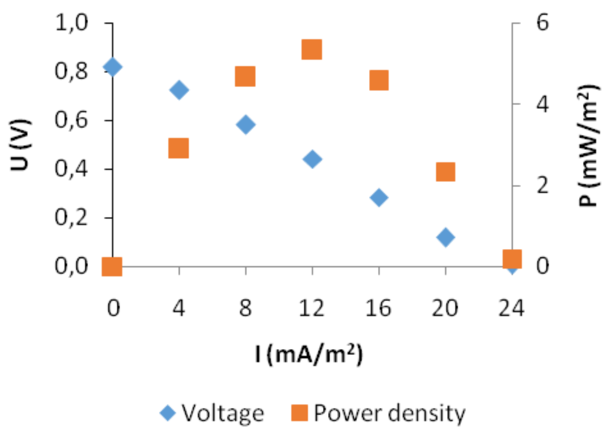

Figure 9.47- 6th polarization curve.

Table 9.54 - Experimental values of voltage vs current density -7th polarization.

7th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.731	0.00
0.01	4	0.635	2.54
0.02	8	0.517	4.14
0.03	12	0.384	4.61
0.04	16	0.226	3.62
0.05	20	0.064	1.28
0.06	24	0.008	0.19
0.03	12	0.384	4.61

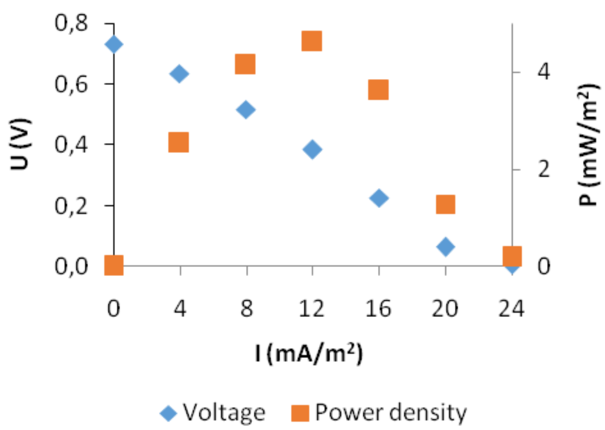

Figure 9.48- 7th polarization curve.

Table 9.55 -MFC conditions for the experimental work.

Test	Reactor	Mode	Anode electrode size	Membrane Area	Membrane	Yeast (mg/L)
10	Single	Batch	BP 1"	25 cm^2	Nafion 117	50

Table 9.56 - Experimental values of voltage vs current density - 1st polarization.

1st Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.659	0.00
0.01	4	0.445	1.78
0.02	8	0.243	1.94
0.03	12	0.051	0.61
0.04	16	0.005	0.08
0.02	8	0.243	1.94

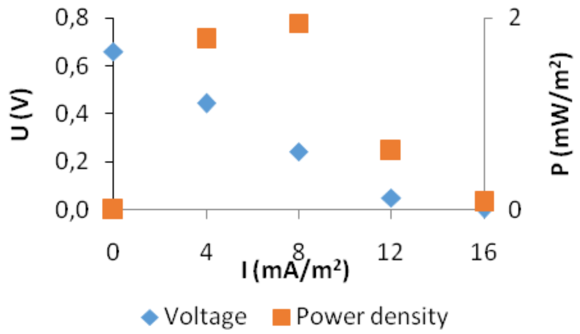

Figure 9.49 - 1st polarization curve.

Table 9.57- Experimental values of voltage vs current density -2nd polarization.

2nd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.689	0.00
0.01	4	0.484	1.94
0.02	8	0.28	2.24
0.03	12	0.09	1.08
0.04	16	0.008	0.13
0.02	8	0.280	2.24

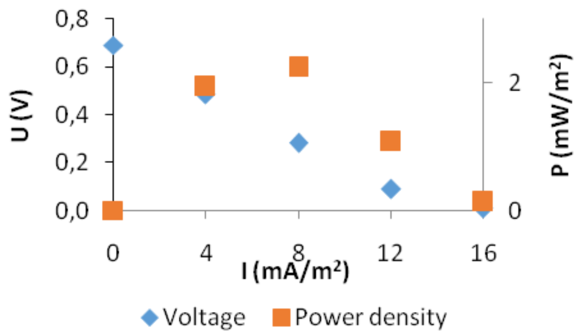

Figure 9.50 - 2nd polarization curve.

Table 9.58 - Experimental values of voltage vs current density -3rd polarization.

3rd Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.739	0.00
0.01	4	0.551	2.20
0.02	8	0.352	2.82
0.03	12	0.171	2.05
0.04	16	0.008	0.13
0.02	8	0.352	2.82

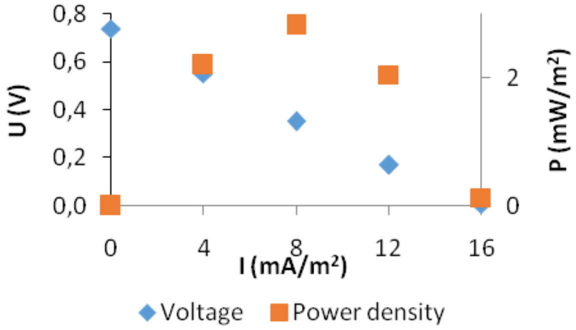

Figure 9.51 - 3rd polarization curve.

Table 9.59 - Experimental values of voltage vs current density -4th polarization.

4th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.732	0.00
0.01	4	0.558	2.23
0.02	8	0.368	2.94
0.03	12	0.190	2.28
0.04	16	0.029	0.46
0.05	20	0.008	0.16
0.02	8	0.368	2.94

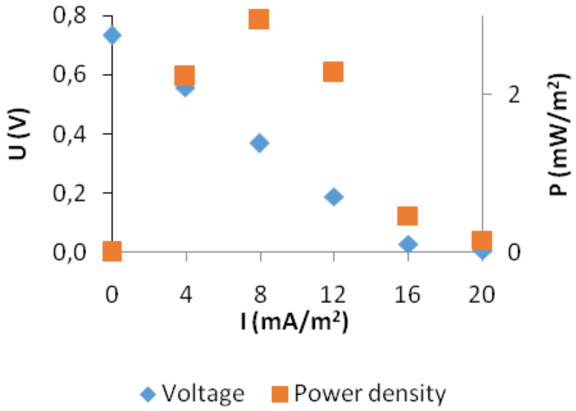

Figure 9.52- 4th polarization curve.

Table 9.60 - Experimental values of voltage vs current density -5th polarization.

5th Polarization			
I (mA)	I (mA/m^2)	U (V)	P (mW/m^2)
0	0	0.727	0.00
0.01	4	0.548	2.19
0.02	8	0.359	2.87
0.03	12	0.197	2.36
0.04	16	0.036	0.58
0.05	20	0.008	0.16
0.02	8	0.359	2.87

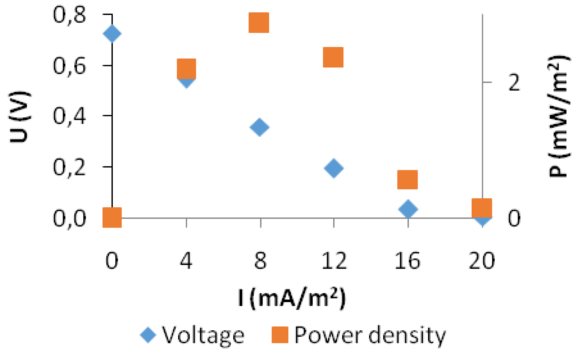

Figure 9.53- 5th polarization curve.

Table 9.61 - Experimental values of voltage vs current density -6th polarization.

6th Polarization			
I (mA)	I (mA/m²)	U (V)	P (mW/m²)
0	0	0.755	0.00
0.01	4	0.590	2.36
0.02	8	0.416	3.33
0.03	12	0.246	2.95
0.04	16	0.094	1.50
0.05	20	0.008	0.16
0.02	8	0.416	3.33

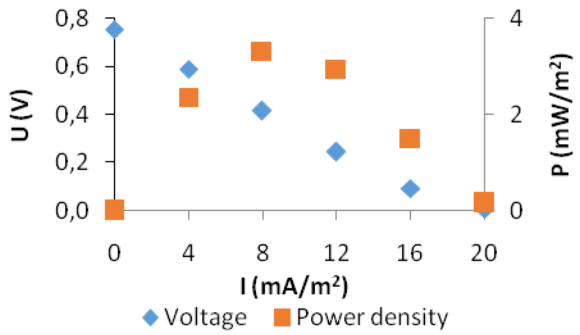

Figure 9.54- 6th polarization curve.

Table 9.62 - Experimental values of voltage vs current density -7th polarization.

7th Polarization			
I (mA)	I (mA/m²)	U (V)	P (mW/m²)
0	0	0.730	0.00
0.01	4	0.571	2.28
0.02	8	0.396	3.17
0.03	12	0.230	2.76
0.04	16	0.079	1.26
0.05	20	0.008	0.16
0.02	8	0.396	3.17

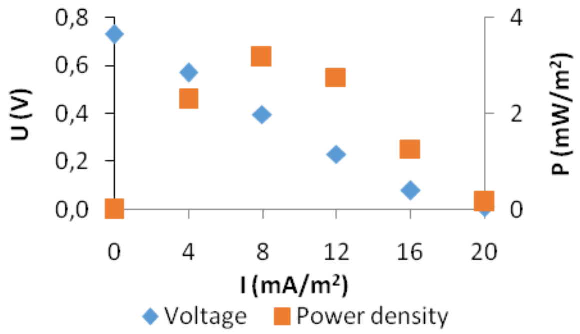

Figure 9.55- 7th polarization curve.